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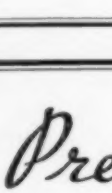

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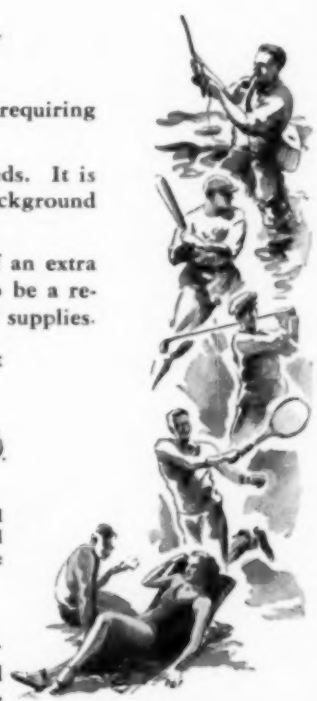

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Comparison of the One- and Eighteen-Hour Frog Method for the Assay of Digitalis

*By C. W. Chapman**

At a conference of those interested in the problems involved in the biological assay of digitalis held at the Atlanta meeting of the American Pharmaceutical Association, 1939, representatives of several laboratories agreed to take part in a collaborative study. The chairman of the U. S. P. Revision Committee undertook to supply the digitalis required for this work. It was suggested that the potency of every lot of digitalis obtained by him be tested biologically before being mixed or treated in any manner for distribution to the collaborating laboratories. Dr. Lloyd C. Miller, of the Food and Drug Administration, and Dr. C. W. Chapman, of the University of Maryland, volunteered to perform these preliminary assays on samples submitted by the chairman of the Revision Committee. The potency of these samples in terms of International Standard, 1936, and the methods used to determine these values are given in this paper.

EXPERIMENTAL

Methods.—Dr. Miller employed the U. S. P. one-hour frog method designing each assay and calculating results by the procedure of Miller, Bliss and Braun (1). The eighteen-hour or over-night method reported by Chapman and Morrell (2) was used by Dr. Chapman. The essential differences in these two methods are:

(*a*) The absorption period in the one-hour procedure is exactly one hour in contrast to the longer period of eighteen hours or "over-night" absorption time of the other method.

(*b*) The end-point in the one-hour method is the condition of the frog's heart (auricles dilated and in standstill, ventricle in systolic standstill). In the over-night method, all dead animals as determined by tactile stimuli are recorded as reacting positively.

(*c*) The method of Miller, Bliss and Braun (1) requires for each substance injected, "standard" or sample, that three different doses be injected into each of three groups of frogs. In the Chapman and Morrell procedure (2) a single dose of "standard" is injected into one group of frogs and one dose of sample into another group of frogs.

(*d*) In the Miller, Bliss and Braun (1) method the slope of the dosage-effect curve b is determined in each assay for "standard" and "unknown." This involves the statistical calculations given by these authors. In the single-dose method the slope of the dosage-effect curve is assumed to be constant or, using Trevan's (3) description, "characteristic" for this particular assay procedure. The characteristic curve referred to in this work is that reported by Chapman and Morrell (4) in 1931, for ouabain, not digitalis. By assuming a constant, b, or slope for the dosage-effect regression line the

* School of Pharmacy, University of Maryland, Baltimore, Maryland.

calculation of the relative potency of a sample of digitalis in terms of a reference standard is extremely easy, involving only a simple proportion.

Standard of Reference.—The International Standard digitalis powder, 1936, supplied by the chairman of the U. S. P. Revision Committee was used for each comparison. A solution containing one International Unit per cc. was prepared by macerating the contents of one ampul of standard (approximately 3 Gm.) with U. S. P. menstruum (approximately 76% ethanol) in the proportion of 0.08 Gm. of standard to each cc. of menstruum and centrifuging. This standard was injected simultaneously with each sample.

Results.—The potency of each sample in terms of International Units per Gm. are compiled in Table I for both one-hour and eighteen-hour methods. It should be emphasized that these figures are the result of routine assays and do not involve the use of a prohibitive number of animals.

Table I.—Digitalis Assays

No. of Sample	Description of Sample	International (1936) Units per Gm. One-Hour Method	International (1936) Units per Gm. Eighteen-Hour Method
A 501-11	Powder	16.3	16.4
B 505-12	Powder	14.2	13.4
C 739	Powder	12.0	11.0
D 509-7	U. S. P. pdr.	15.3	15.2
E 503-5	I. S. D. pdr.	13.3	12.5
F 504-14	Leaf	11.2	8.3
G 510-15	Leaf	11.8	15.8

DISCUSSION

The agreement between the potency of the first five samples (*A*, *B*, *C*, *D*, *E*) as determined by both one-hour and eighteen-hour methods is very good. The maximum variation from the official one-hour method is approximately 5%, which is well within the error to be expected from the standard deviation of the results. Samples *F* and *G* were whole- or crushed-leaf specimens. The agreement between the assays for these is not as close as for the other samples. This is quite possibly due to the difficulty in properly sampling a crude drug which consists of whole leaves or parts of large dimensions. It emphasizes the fact that much care must be exercised in taking such samples.

Sample *E* is a sample of the 1936 International Standard which was identified only by a number and included with the other batches to be assayed against another portion of this same standard. The labeled potency of this standard is 12.5 International Units per Gm. (one International Unit = 0.08 Gm.). By the eighteen-hour method the determined potency was exactly 12.5 units, by the one-hour method 13.3 units. These values are identical within the limits of error of the method and are the same as the labeled potency for this standard.

Sample *D*, the current U. S P. Reference powder possesses 15.25 International (1936) Units per Gm. determined by taking the average of both methods, 15.3 and 15.2 units. One gram of the U. S. P. Reference powder is equivalent to 15.25 International Units when the International (1936) Standard is considered to possess one International Unit per 0.08 Gm., or 0.0656 Gm. U. S. P. Reference Standard contains 1 International (1936) Unit. Both one-hour and eighteen-hour methods give this equivalent. This is not in accord with the official factor of 0.0745 which appears on the label of the U. S. P. Reference Standard. Referring to the official one-hour and the "over-night" methods, Edmunds, Moyer and Shaw (5) state that "with different periods of observation the potencies of different powders in relation to a standard vary considerably." The results reported here do not confirm this statement. Both methods give the same relationship within the limits of error for each of the powders assayed (*A*, *B*, *C*, *D*, *E*) in comparison with the International (1936) Standard.

It can be argued that the characteristic curve for the reaction of frogs to digitalis will vary from time to time or from one batch of frogs to another when every precaution is taken to keep the conditions of assay constant. There is also the possibility that samples of digitalis are from different sources, of different age or prepared by different methods. This may result in significantly different values for *b* when tested under identical conditions. Calculations for the one-hour method (1) used in this work include the determination of *b* for every assay in order to eliminate any of these possible variations. However, that the introduction of a predetermined characteristic curve or *b* value does not affect the results appreciably is demonstrated in the eighteen-hour method results. Elimination

of the determination of b greatly simplifies the calculations. If a standard or characteristic curve, such as that employed in this work, can be used in the assay of digitalis (4) it is to be preferred because of the simplicity in performing an assay and calculating results.

CONCLUSION

1. Seven samples of digitalis, powder and leaf, are compared by the one-hour and eighteen-hour methods. Good agreement was observed.

2. The U. S. P. Reference powder possesses 152.5% of the potency of the 1936 International Standard when the latter is considered to possess an activity of one International Unit per 0.08 Gm.

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Progress Report on the U. S. P. (1939–1940) Digitalis Assay Study*

By Lloyd C. Miller

As a result of a conference on the Assay of Digitalis held in connection with the Atlanta Meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION, a collaborative study of the assay of digitalis using frogs has been in progress during the past year. The program is a U. S. P. project made possible by the coöperation of the participating laboratories. As a consequence of my offer to calculate and compile the results of the study, it now falls to my lot to serve as a narrator in presenting a summary of the progress to date.

* Report prepared at the request of Dr. C. W. Chapman, Chairman, A. PH. A. Committee on Physiological Testing, with the approval of Dr. E. E. Nelson, Chairman, Committee on U. S. P. (1939–1940) Digitalis Assay Study.

EXPERIMENTAL

The conference decided that the U. S. P. reference digitalis powder was unsuitable for use in this collaborative program and requested Professor Cook to collect immediately samples of both domestic and imported digitalis leaves of good quality to be composited as study material for this project. As a result 110 lb. were collected from various sources and upon the very prudent suggestion of Dr. C. W. Chapman, who had had considerable experience in compositing the present Canadian digitalis standard powder, each of these samples of leaves was subjected to bioassay. The findings in these assays have just been reported by Dr. Chapman.

It was the consensus of the Atlanta conference that probably the most important issue was the question of the alleged superiority of the so-called "over-night" frog method over the official U. S. P. one-hour method. Of interest also was the most suitable means of preparing an extract of a standard powder prior to assay, *i. e.*, a hot exhaustive extraction as compared with a cold maceration procedure. Then and in subsequent correspondence the cat method was considered, but it was decided finally to seek to perfect one method at a time.

It was felt that the details of preparing a macerate could be specified rigidly enough to insure a preparation sufficiently uniform to enable a valid comparison between the one-hour and eighteen-hour methods. It is to be noted that the term "eighteen-hour" was applied to designate the so-called "over-night" method, a decision which was indicative of the resolve of the conference to make a fresh start. It was freely expressed at the conference that great care should be exercised toward devising a workable and well-defined plan for the assays. It may now be said that this desire was thoroughly justified.

THE FIRST COMPARISON

It was decided that the program should be made up of a series of what may be termed major comparisons, each of which would bear directly on a well-defined and vital phase of the problem. Thus the first comparison was designed to determine (1) the relative merits of the one- and eighteen-hour methods when conducted under as nearly identical conditions as practicable and (2) the practicability of diluting a standard powder with exhausted marc. Toward this end two samples of digitalis powder, Samples 1 and 2, were submitted to nearly 20 laboratories. One of these powders was a dilution of the other, the diluent being thoroughly exhausted marc (the inactivity of which was proved biologically) in a proportion known to none of the collaborators. Each collaborator was requested to determine the relative potency of these two samples by both the one- and eighteen-hour methods under conditions quite rigidly specified.

The data for the one-hour and eighteen-hour methods were compared on the basis of four criteria which were proposed in a paper presented to the

Scientific Section of the AMERICAN PHARMACEUTICAL ASSOCIATION at Atlanta and published in the October 1939 number of the JOURNAL. As a result of this objective comparison, certain unmistakable advantages appeared to be attached to the eighteen-hour method. One of these is indicated in the following table which gives the results submitted on the first comparison.

Results of First Comparison—U. S. P. Digitalis Study

Values indicate potency of Sample 2 in terms of Sample 1 and are weighted means ± the standard errors obtained by each laboratory. Figures in parentheses indicate number of assays if other than two.

Laboratory Number	One-Hour Method, Per Cent Potency	Eighteen-Hour Method, Per Cent Potency
1221	122 ± 8 (3)	126 ± 6 (3)
1223	130 ± 13	148 ± 9 (1)
1226	134 ± 10	152 ± 12
1227	130 ± 18	126 ± 12 (1)
1228	148 ± 11	130 ± 4
1229	127 ± 8 (4)	144 ± 6 (4)
1235	144 ± 12 (1)	142 ± 6 (1)
1237	172 ± 61 (1)	
1238	120 ± 7	143 ± 6
1241	165 ± 25 (1)	163 ± 17 (1)
Weighted mean—all laboratories	130.9 ± 3.4	138.4 ± 2.3

Actual relationship, 140.0 per cent

THE SECOND COMPARISON

It was decided that the second comparison should be planned to supply data on the reproducibility of a hot extraction procedure as compared with cold extraction (maceration). It became clear at once that if assays were to be made upon extracts prepared by two different methods from a single powder and only these two variables were studied, there would be no way of associating any variability observed with one method or the other. That is, while we assumed in the first comparison that a perfectly uniform liquid preparation could be prepared by all collaboratores by the maceration technique, this assumption was not valid in the present case. Consequently, it was decided to provide a 4-oz. sample of tincture as a standard against which the macerate and the hot extract could be compared. Thus a tincture and a sample of the powder from which it was prepared were then submitted to each collaborator.

Wherever comparable, the results submitted thus far on the Second Comparison bear out the data submitted earlier. The principal features of the data as a whole are (1) the remarkable uniformity in the potencies of macerates prepared from the same digitalis powder in different laboratories and (2) the very low error of the eighteen-hour method.

Russia has issued a postage stamp in honor of Dimitri Ivanovich Mendeléjeff for his outstanding work as a scientist.

Barbituric Acid Derivatives*

Relationship between Hemolytic Action and Chemical Structure

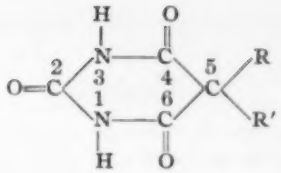
By Henry M. Lee and Edward E. Swanson

In a previous communication (1), it was observed that there is an obvious relationship between the pharmacological action and the chemical structure of certain barbituric acid derivatives. In the primary or secondary alkyl-substituted compounds, with an increase in the number of C-atoms in the alkyl group, both the minimal anesthetic dose (M. A. D.) and the minimal lethal dose (M. L. D.) grow relatively smaller, but when the alkyl radical is longer than 5 C-atoms, the amount required to produce anesthesia or death in rats again increases. As the alkyl chain lengthens, the therapeutic index or ratio between M. L. D. and M. A. D., appears to be gradually greater. In general, the duration of action shows similar features; that is, it is shorter when the alkyl group becomes lengthened. In normal alkyl-substituted derivatives, the critical compound is the one that possesses 6 C-atoms in one of the 5–5 positions; in the secondary alkyl-substituted derivatives, the critical compound is the one having 7 C-atoms in one of the 5–5 positions, beyond which the duration of action begins to increase. More recently, it has been reported that the substitution of a methyl, ethyl or allyl radical on the nitrogen (nitrogen alkyl-substituted barbituric acid derivatives) (2), or the substitution of an alkyl, methallyl (2-methyl allyl) (3), or crotyl (3-methyl allyl) (4) on one of the 5–5 positions, or a sulfur atom (5) in place of the oxygen on the 2 C-atom obviously reduces the duration of action. This shorter duration of action is independent of the amount of drug administered.

EXPERIMENTAL

The present investigation deals with the study of the relationship between the hemolytic action on the red blood cells and the chemical structure of certain barbituric acid derivatives synthesized by Shonle and his associates of this laboratory, with the general formula:

* From the Lilly Research Laboratories, Indianapolis, Indiana.



wherein R denotes an alkyl radical (normal or secondary with 2 to 8 C-atoms) and R′ an ethyl radical. A number of thiobarbituric acid derivatives were also studied, wherein R′ indicates crotyl (3-methyl allyl) and R an alkyl radical (normal or secondary with 2 to 5 C-atoms).

The apparatus used in testing the hemolytic action was a modification of that described by Ponder (6). Small test-tubes containing the hemolytic system were suspended in a glass water-bath maintained at a constant temperature (37.7° C.). Each test-tube contained 0.4 cc. of a suspension of thrice-washed sheep blood cells, representing 0.05 cc. of whole sheep blood plus 1.6 cc. of a 0.05*M* barbituric acid sodium salt solution and 0.154*M* sodium chloride. The hydrogen-ion concentration of each suspension was adjusted to p_H 10.

As shown in Chart 1, certain barbituric acid derivatives caused the blood to hemolyze. The hemolytic time was dependent on the number of C-atoms in the substituted alkyl radical. In the primary alkyl substituted compounds, diethyl barbituric acid (2 C-atoms in the alkyl radical) produced hemolysis in 288 minutes. As the alkyl radical increased in length from 2 to 7 C-atoms, there was a rapid decrease in the hemolytic time from 288 minutes to less than 1 minute. In the secondary alkyl-substituted barbituric acid derivatives, with the exception of the secondary propyl (3 C-atoms), the increase in the number of C-atoms on the alkyl radical from 4 to 8 lowered the hemolytic time from 260 minutes to 1 minute. The primary and secondary alkyl-substituted derivatives of the alkyl crotyl thiobarbituric acid derivatives showed an even greater decrease in the hemolytic time. As the number of C-atoms increased in the alkyl radical from 2 to 5, the hemolytic time decreased from 348 minutes to 6 minutes. Thus, there appears to be a definite relationship between the hemolytic action on the red blood cells and the chemical structure of certain barbituric acid derivatives.

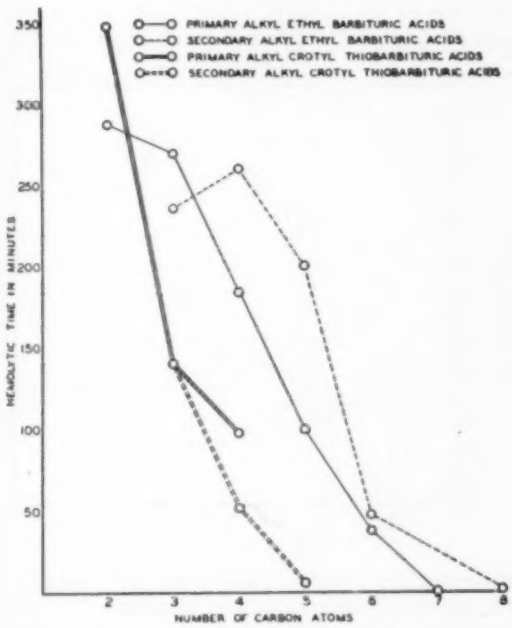


Chart 1.—Hemolytic Time of Barbituric Acid Derivatives.

The authors are indebted to Dr. K. K. Chen, Director of Pharmacologic Research, for his valuable suggestions and criticisms and to Dr. W. W. Davis for his technical assistance.

SUMMARY

1. A series of primary and secondary alkyl-substituted barbituric acid and thiobarbituric acid derivatives were studied for their hemolytic action on the sheep's blood.

2. The hemolytic time decreases as the number of C-atoms increases in the substituted alkyl radical.

3. There is obviously a relationship between the hemolytic action and the chemical structure of certain barbituric acid derivatives.

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The following scientists have been honored by France by portrayal on postage stamps: Marcelin Berthelot, pioneer in organic synthesis; Claude Bernard, physiologist; Louis Pasteur, Pierre Curie and Marie Curie, discoverers of polonium and radium; Leon Calmette, research worker in tuberculosis.

The Use of Sodium Pentobarbital for Repeated Anesthesia in the Guinea Pig

*By V. Everett Kinsey**

The first paper of this series (1) dealt with the use of sodium pentobarbital (Nembutal)[1] for repeated anesthesia in the rabbit. The present study will describe the problems which arise from the use of this drug for repeated anesthesia in the guinea pig. Rather oddly, there have been few studies of the use of sodium pentobarbital for this animal. Carmichael and Posey (2) report that, after six bi-weekly intraperitoneal injections of 15 mg. per Kg. of Nembutal, guinea pigs showed a definite tolerance to approximately 18 mg. per Kg. of this drug as measured by the sleeping time. Daily injections (period not given) produced tolerance much faster, but in no case was the M. F. D. (minimum fatal dose, *i. e.*, amount killing 90 per cent of the guinea pigs) increased as a result of previous injection. These authors (3) also studied the M. L. D. and found it to vary from 45 mg. per Kg. for 200-Gm. guinea pigs to 60 mg. per Kg. for 800-Gm. guinea pigs.

MATERIALS AND METHODS

Young guinea pigs were kept in groups of five in large wire-bottom cages. Their diet consisted of oats, bran and hay daily with greens from two to four times weekly, and some butterfat and milk powder once a week. While the Nembutal solution was but one-tenth so concentrated (6.5 mg. per ml. of 0.9 per cent NaCl containing 10 per cent ethyl alcohol) as that used in the previous study (1), the procedure for administering the intraperitoneal injections to the guinea pigs was identical with that used for the rabbits. In view of the variation of response with the weight all comparative studies were made with animals of the same weight group. The sleeping time was taken as the period during which the righting reflex was lost.

EXPERIMENTAL

In view of the fact that a possible synergistic effect of the alcohol, used in making up the solution of Nembutal, would materially alter the interpretation of the results to be reported below, the influence of the alcohol was first investigated. The sleeping time of 8 guinea pigs, given 15.6 mg. per Kg. of Nembutal in a solution containing 10 per cent alcohol (0.2 Gm. per Kg.), averaged 86 minutes, as compared to 88 minutes in a control group of 8 animals which received the same dose of Nembutal but no alcohol. None of these animals was injected before.

The above finding was confirmed by the following experiment: 40 small (200–300 gram) guinea pigs, which had not been injected previously, were divided into 8 equal-sized groups. Each group of animals was then injected with 15.6 mg. per Kg. of Nembutal, dissolved in 0.9 per cent NaCl and increasing amounts of alcohol. It may be seen from Fig. 1, where the average sleeping times resulting from these injections are plotted, that the sleeping time does not appear to be increased appreciably until at least 0.4 Gm. per Kg. of alcohol is given simultaneously with the Nembutal. This is twice the amount of alcohol given in any of the experiments which are to be described later.

It is of interest to compare the above results with the initial, greatly increased, sleeping time of the rabbit (1) with doses of alcohol as low as 0.055 Gm. per Kg.

It was observed, however, that considerable tolerance was established in the rabbit after one injection of the alcoholic Nembutal. Since some potentiation was observed above 0.5 Gm. per Kg.

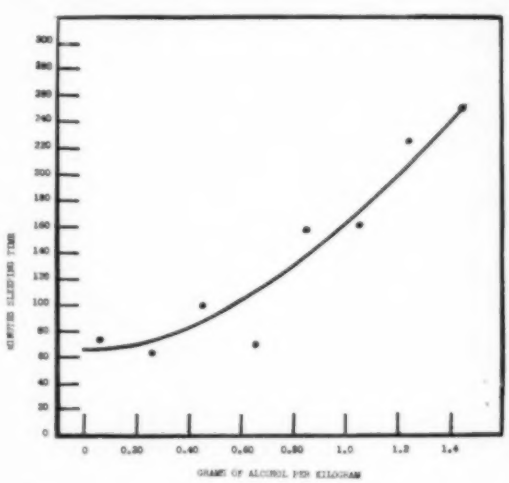

Fig. 1.—Showing the average sleeping time of 8 groups of 5 guinea pigs each, following an intraperitoneal injection of 15.6 mg. per Kg. of Nembutal and increasing quantities of ethyl alcohol.

* Department of Physiological Chemistry, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

[1] Kindly supplied by the Abbott Laboratories, North Chicago, Ill.

of alcohol in the guinea pig it was decided to find whether the guinea pig, too, would exhibit tolerance when given a second dose of the Nembutal and alcohol. Accordingly, the experiment described above with the 8 groups of 5 animals each was repeated exactly after an interval of one week. The sleeping times for the 8 doses of alcohol were then found to be 108, 112, 108, 123, 121, 137, 251 and 243 minutes, respectively. These data indicate that no tolerance to the alcohol is evident, at least following a week's rest period, and, furthermore, they confirm the results of the first injections, *viz.*, that over 0.4 Gm. of alcohol per Kg. is necessary before any synergism is observed between the alcohol and Nembutal.

A standard dose (15.6 mg. per Kg.) of Nembutal was now given to 6 guinea pigs daily, and to 6 other guinea pigs on alternate days for a period of 19 days. The sleeping times were recorded. (See curves, left-hand part of Fig. 2.) Then because all of the guinea pigs used for long-term studies (described below) were fed the Sherman, La Mer and Campbell (4) vitamin C free diet, supplemented with pipetted feedings of ascorbic acid, the above results were compared to those obtained by repeating the experiment on the same animals two weeks later. During this interval the animals were placed on the special diet. (See curves, right-hand part of Fig. 2.) At the start of the second series of Nembutal injections, the diet was supplemented each day with a maintenance dose of 0.5 mg. of ascorbic acid for each guinea pig.

The solid lines in Fig. 2 refer to the group of animals receiving injections daily, while the dotted lines refer to the group which were injected on alternate days. Like rabbits, following the first several anesthesias, the guinea pigs exhibit an appreciable amount of tolerance which depends upon the frequency of administration. (It will be shown later, however, that when anesthesias are repeated over sufficiently long periods of time the guinea pigs increase significantly in weight, their susceptibility to Nembutal again increases and less drug is required to make them sleep for a given period.) A comparison of the right- and left-hand curves in Fig. 2 shows that the special diet, containing maintenance levels of ascorbic acid, appears to increase the sleeping times slightly; however, more animals would be required to establish the significance of this effect.

Having determined the length of time guinea pigs would sleep as a result of repeated injections of a constant dose of Nembutal, the dosage was next varied in such a way that the length of sleeping time was kept as nearly constant as possible. A period of two hours was chosen for this period of anesthesia.

Two groups of guinea pigs, *A* and *B*, containing 6 and 4 animals, respectively, were maintained on the Sherman diet supplemented with 0.5 mg. of ascorbic acid daily. These animals were injected with Nembutal every other day, excepting Saturday and Sunday, over a period of about 6 months. Throughout this period 4 of the animals of Group *A* died of respiratory collapse at the time of injection, while all of those of Group *B* survived.

The dose required each day to produce anesthesia for the 2-hour period is shown in Fig. 3. Because it was obviously impossible to produce anesthesia for

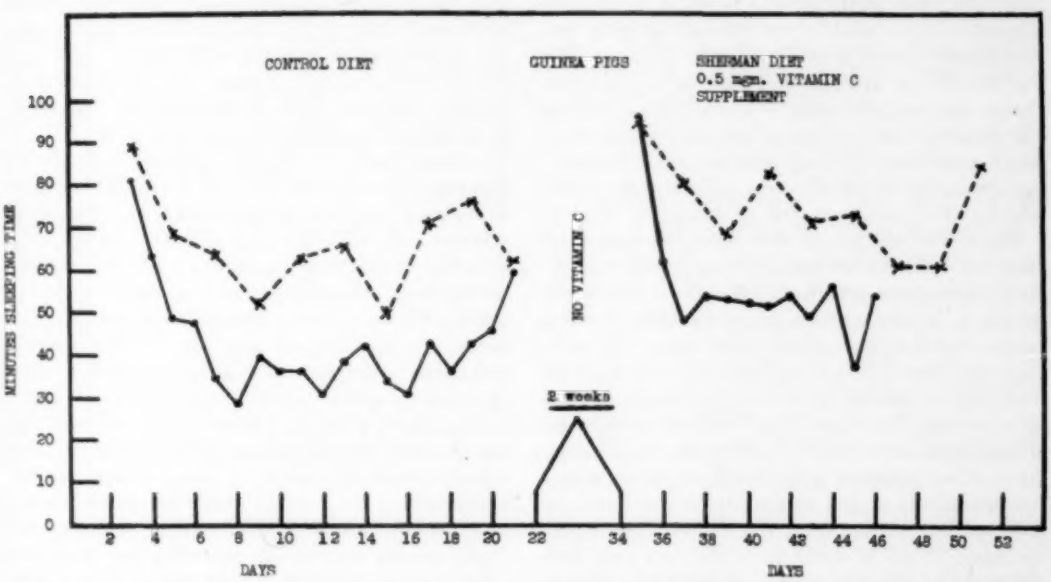

Fig. 2.—Curves on the left represent the average sleeping times of 6 guinea pigs injected daily (solid line) and 6 guinea pigs injected on alternate days (broken line) with 15.6 mg. per Kg. of Nembutal while maintaining the animals on a completely adequate diet. Curves on the right represent the same animals, treated in the same manner, but maintained on the Sherman diet supplemented with 0.5 mg. of ascorbic acid per animal per day. See text for explanation.

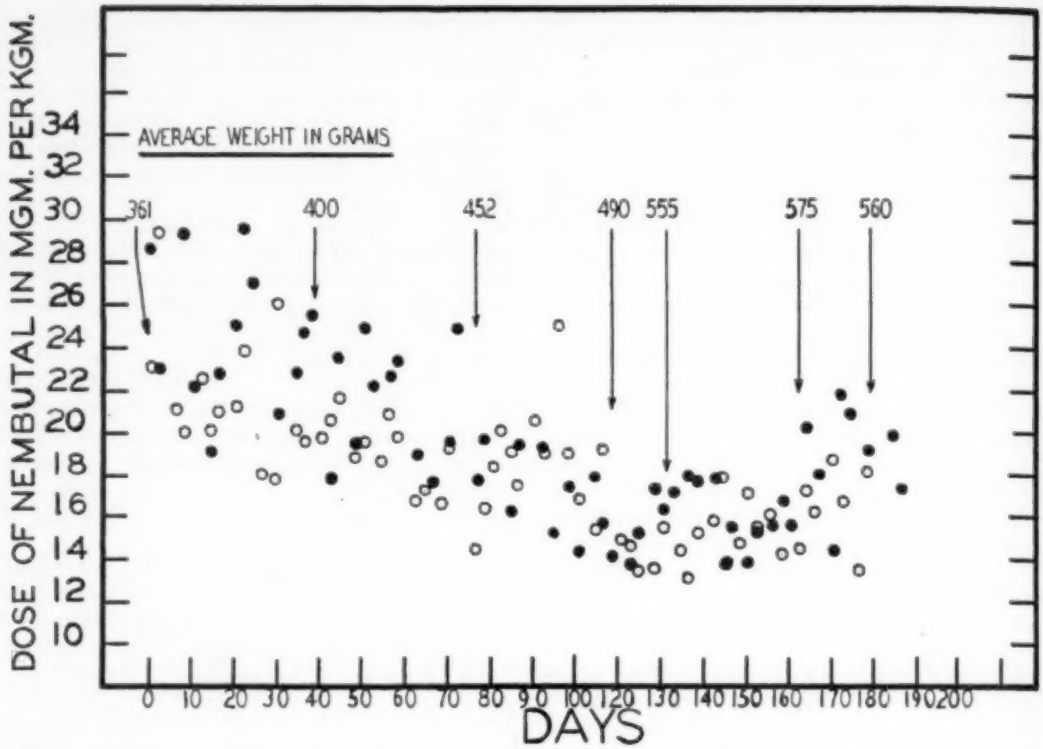

Fig. 3.—Showing the dose of Nembutal required to produce repeated anesthesia in guinea pigs for 120 minutes. Group A Open Circle—6 animals; Group B Solid Circle—4 animals.

precisely the 2-hour test period a small correction had to be made in the dose to adjust it up or down depending upon whether the average sleeping time was slightly above or below the 120 minutes. For example, if the average sleeping time on any particular day was 110 minutes instead of the desired 120 minutes the additional amount of Nembutal which would have been required to increase the sleeping time 10 more minutes was estimated from data which will be presented later in this paper (Fig. 4).

Figure 3 shows that the dose necessary to produce sleep for 120 minutes appears to decrease progressively throughout practically all of the experimental period, *i. e.*, the animals gradually became more, rather than less, susceptible to the drug. Of interest is the very close inverse relationship between the dose and the average weight of the animals. It will be seen from the figure that while the weight increases regularly up to the 150th day, the dose decreases, but following a rather abrupt increase to a nearly constant weight, due in part to the death of a 360 Gm. animal, the dose, too, remains practically constant. Thus it would seem that not only does the M. L. D. as noted by Carmichael and Posey (3) decrease with heavier (or older) guinea pigs but so does the sleeping time. It should be noted that no difference in the results would have been obtained if the dose had been based on area of the animal as suggested by Moore (5) instead of the weight. It would appear that both dosage on a weight basis and dosage on an area basis must still be considered arbitrary in the case of Nembutal and guinea pigs. In contrast when working with rabbits the ratio of the dosage to the sleeping time was found to remain constant when Nembutal was given repeatedly over a period of almost a year.

Having studied the dosage required to produce a particular sleeping time, *i. e.*, 2 hours, the response to varying doses was next investigated. The doses selected were 15.6, 25, 31.3 and 37.5 mg. per Kg. In a few instances one guinea pig would be used a second time, but a rest period of at least a week was allowed between tests. (No differences in sleeping times were detected in such cases.) The average and range of sleeping times for the animals in these experiments appear under "Single Dose" in Table I and graphically in Fig. 4 where it may be seen that the sleeping time increases linearly with the dose when a threshold amount of about 10 mg. per Kg. is exceeded. The average weight of the guinea pigs was 350 Gm. with an extreme range of 300 to 600 Gm. Of the 51 animals receiving the first dose (15.6 mg. per Kg.) the sleeping time was slightly lower for the 33 weighing less than 350 Gm. than for the 18 which weighed more than this amount. Considering the wide individual variations encountered, these differences (86 *versus* 96 minutes) may not be significant.

Table I.—Comparison Between the Lengths of Sleeping Time of Guinea Pig When Injected with as Single Dose of Nembutal and When the Same Amount Is Given in Two Separate Injections

No. of Guinea Pigs	No. of Groups	Dose (Injections) First + Second = Total mg./Kg.	Average Sleeping Time, Min.	Range of Average Sleeping Time of Each Group, Min.	Range of Individual Sleeping Time, Min.
		Single Dose			
51	7	15.6	89.4	75–115	35–155
25	4	25	201	193–206	95–338
6	1	31.3	286	...	195–332
21	4	37.5	352	336–375	244–455
		Double Dose			
10	2	15.6 + 9.4 = 25	239	229–246	181–285
6	1	15.7 + 15.6 = 31.3	269	...	220–305
10	2	15.6 + 21.9 = 37.5	365	344–385	304–457
9	2	25 + 12.5 = 37.5	365	356–375	273–458

Table II.—Showing the Lengths of Sleeping Time of Male and Female Guinea Pigs When Injected Intraperitoneally with 15.6 mg. per Kg. of Nembutal

Sex	Number of Guinea Pigs	Average Sleeping Time, Min.	Range of Sleeping Time, Min.
Males	25	92.4	35–190
Females	21	84.0	35–130

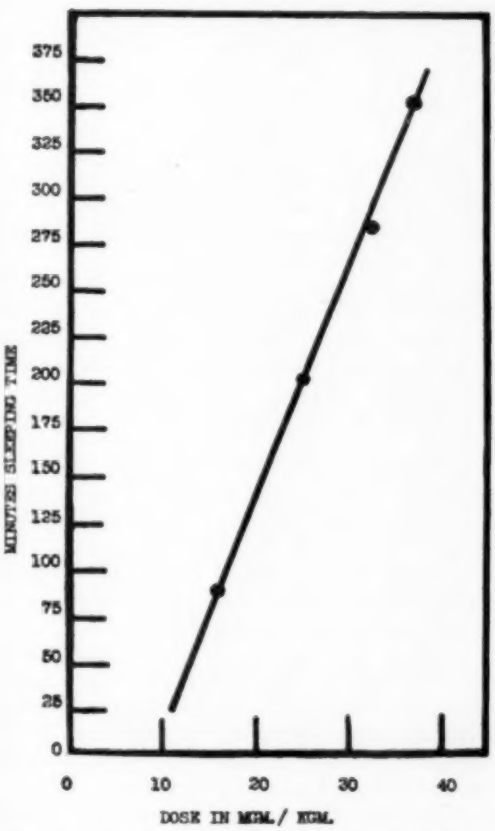

Fig. 4.—Showing the sleeping time resulting from increasing doses of Nembutal.

It had been found (1), particularly in the case of rabbits, that when longer sleeping times were desired it was necessary for the safety of the animals to give a second and sometimes a third dose of Nembutal. This raised the question of the comparative sleeping time of divided doses, as against giving the total amount or drug in a single injection. The guinea pig provides an excellent test animal for this purpose because of the wide range between the anesthetic and lethal dose. The three higher doses used previously, 25, 31.3 and 37.5 mg. per Kg., respectively, were each divided into two doses. The first injection was given and, immediately after the animal regained consciousness, the second dose was administered. It will be seen from the lower half of Table I that the total sleeping time is about the same whether the drug is given all at once or in two doses.

Lastly, the response of male and female guinea pigs, as judged by the sleeping time, was tested. A dose of 15.6 mg. per Kg. of Nembutal was given to 25 male and 21 female animals. The average sleeping times and range of the sleeping times given in Table II show that there is little difference between the two sexes. The average weights of the two groups were 338 and 366 Gm. for male and female animals, respectively.

CONCLUSIONS

1. It has been shown that ethyl alcohol does not potentiate the action of Nembutal in guinea pigs as measured by increased sleeping time, unless given in amounts in excess of 0.4 Gm. per Kg. with doses of Nembutal of 15.6 mg. per Kg.

2. The sleeping time of guinea pigs subjected to repeated anesthesia with 15.6 mg. per Kg. of Nembutal decreases for the first several daily injections. Similarly treated guinea pigs maintained on a low level of ascorbic acid appear to sleep about 10 per cent longer than those given an adequate supply of greens.

3. When anesthesia of 2 hours' duration is produced every other day over a six-month period by giving intraperitoneal in-

jections of Nembutal, the susceptibility of the animals to this drug gradually increases. This increase appears to be associated with an increase in the weight of the animals.

4. The sex of the guinea pig did not appear to alter the sleeping time following single injections of Nembutal.

The author wishes to express his appreciation to Dr. Charles F. Kutscher through whose liberal support this study was made possible.

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Potassium Bismuth Saccharate. II. Toxicity, Absorption and Distribution of Bismuth Following Intramuscular Injection*

By C. W. Sondern, A. E. Pugh, F. V. Kalich, George Lann and C. J. W. Wiegand

The preparation and chemical properties of a water-soluble potassium bismuthyl saccharate have been given in a previous report from this laboratory (1). This paper presents a study of the absorption, distribution, excretion and toxicity of a preparation containing this compound.[1]

EXPERIMENTAL

The absorption, distribution and excretion in rabbits was determined after a single intragluteal injection of doses equivalent to 5 mg. and 3 mg. of bismuth per Kg. body weight into two groups of six animals each. These doses were as small as the precision of the analytical method would permit for subsequent determination of the distribution of the bismuth in the various organs. One, three and five days after the injection, two animals in each group were sacrificed by exsanguination and the organs immediately removed for assay. The excretion was determined by collecting the urine and feces separately after each twenty-four-hour interval.

Quantitative determinations of the bismuth content of the biological material were made by the iodide method for amounts of the metal in excess of fifty micrograms (2), and by the dithizone method for smaller concentrations (3).

Table I presents the data on total excretion of bismuth in terms of the per cent of the amount injected.

Table I.—Excretion in Per Cent of Amount of Bismuth Injected

Dose, mg./Kg.	Rabbit No.	Excretion Time, Days	Urine	Feces	Total
5	1	1	6.0	0.7	6.7
	2		0.8	0.1	0.9
	3	3	1.2	0.5	1.7
	4		8.1	4.7	12.8
	5	5	11.6	3.7	15.3
	6		17.4	6.5	23.9
3	7	1	1.2	0.1	1.3
	8		0.3	0.1	0.4
	9	3	13.1	0.2	15.1
	10		15.6	2.0	17.6
	11	5	16.1	2.6	18.7
	12		23.3	4.1	27.4

It is seen that with the larger dose of bismuth about one-fifth of the amount injected is excreted within the five-day period with the peak of the excretion occurring after the first three days. With the smaller dose a disproportionately smaller amount was found in the excreta. The daily variation in the amount found was roughly proportional to the quantity of excreta but individual animals showed no constant relationship between the amount of bismuth excreted and the time after injection.

Table II shows the distribution of the bismuth in the organs of the injected animals. In addition the last column gives the total recovery of bismuth in terms of the per cent of the injected quantity.

The toxicity of potassium bismuthyl saccharate for white rats by both intravenous and intramuscular injection is shown in Table III.

Sections of the kidneys of the animals receiving a fatal dose of the bismuth compound showed widespread pathology. The rapidly fatal doses produced desquamation of the epithelium of the convoluted tubules, dilatation of the tubules and abundant albuminous casts. Animals living longer than one week but subsequently succumbing to the metal showed, in addition to the above changes, focal areas of necrosis of the kidney parenchyma and more or less extensive calcification of the tubular epithelium. The animals receiving a sub-lethal dose showed, after three weeks' observation, cloudy swelling and some desquamation of the tubular epithelium.

* From the Research Laboratories of George A. Breon & Co.

[1] Sacbimuth—a solution containing 50 mg. potassium bismuthyl saccharate per cc. (equivalent to 25 mg. bismuth), 25 per cent sucrose and 2 per cent benzyl alcohol.

Table II.—Distribution of Bismuth in the Organs
(Mg. of Bi/100 Gm. of Organs)

No.	Time, Days	Kidney	Liver	Colon	Muscle	Heart	Spleen	Central Nervous System	Blood Cells	Blood Serum	Inj. Leg	% Recovery
		Dose—5 mg. of Bi per Kg.										
1	1	2.25	0.37	0.38	0.06	0.003	0.02	0.001	0.001	0.003	8.42	93
2	1	1.83	0.30	0.40	0.05	0.003	0.04	0.003	0.001	0.002	7.87	88
3	3	2.80	0.13	0.31	0.06	0.003	0.02	0.002	0.001	0.004	4.73	77
4	3	1.60	0.20	0.06	..	0.012	0.05	0.004	0.001	0.006	2.83	67
5	5	2.00	0.10	0.06	0.02	0.006	0.02	0.004	0.002	0.002	5.09	54
6	5	3.11	0.15	0.06	..	0.003	0.02	0.003	0.001	0.005	8.09	87
		Dose—3 mg. of Bi per Kg.										
									Total Blood			
7	1	0.90	0.25	0.10	0.03	0.008	0.04	0.002	0.002		3.50	92
8	1	1.50	0.41	0.13	0.02	0.004	0.02	0.004	0.001		3.28	90
9	5	3.00	0.11	0.06	0.06	0.012	0.05	0.003	0.003		2.40	84
10	5	2.20	0.08	0.03	0.05	0.006	0.03	0.003	0.004		2.45	83

Table III.—Toxicity of Potassium Bismuthyl Saccharate

Intravenous

Dose mg. of Bi per Kg.	No. of Animals	Per Cent Deaths	L. D.$_{50}$
7.0	9	0	10.41 mg. of Bi/Kg.
8.0	9	0	
9.0	16	0	
9.5	10	30	
10.0	20	35	
11.0	5	100	
12.0	3	100	

Intramuscular

Dose mg. of Bi per Kg.	No. of Animals	Per Cent Deaths	L. D.$_{50}$
75.0	3	0	172.1 mg. of Bi/Kg.
100.0	5	0	
120.0	10	0	
140.0	10	10	
150.0	10	10	
170.0	14	50	
200.0	6	100	
250.0	3	100	

SUMMARY

1. Excretion in Rabbits.—(*a*) With a single dose of 5 mg. Bi/Kg. 15–24% of the injected bismuth is excreted within five days. With the smaller dose of 3 mg. Bi/Kg. 19–27% of the injected bismuth is excreted in the same period of time.

(*b*) The ratio of the bismuth excreted in the urine to that excreted in the feces is 3:1 with the larger dose and about 6:1 with the smaller.

(*c*) The peak of excretion lies between the third and fifth days.

The excretion of bismuth after intramuscular injection of potassium bismuthyl saccharate solution is somewhat slower than has been reported for other water-soluble preparations (4).

2. Absorption.—(*a*) The absorption from the site of injection appeared to be gradual but somewhat variable within individual animals. With the larger dosage, 26% was absorbed on the first day, 60% within three to five days. On the smaller dosage, 63% was absorbed the first day and 70% within five days.

The absorption of the potassium bismuthyl saccharate is somewhat slower than the generally reported absorption of other water-soluble bismuth preparations (4).

3. Distribution.—(*a*) The kidney shows the greatest concentration of the bismuth (1.00–3.11 mg./100 Gm.).

(*b*) The liver is next in bismuth content varying from 0.08 to 0.41 mg./100 Gm.

(*c*) The bismuth content of the colon varies with the quantity of fecal contents.

(*d*) Heart—3–12 micrograms in heart muscle.

(*e*) Spleen—20–50 micrograms.

(*f*) Central nervous system—1–4 micrograms.

(*g*) Blood cells—1–2 micrograms; blood serum—1–6 micrograms.

4. Toxicity.—Intravenous—10.41 mg./Kg.; intramuscularly—172.0 mg./Kg.

The intravenous and intramuscular toxicities of the potassium bismuthyl saccharate are comparable to those reported for the tartrate (4).

CONCLUSIONS

Intramuscular injections of potassium bismuthyl saccharate are gradually absorbed and excreted. Bismuth is present in all the organs and tissue fluids within 24 hours resulting in fairly uniform blood bismuth levels for 5 days.

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Effect of Fresh *Alœ Vera* Jell in the Treatment of Third-Degree Roentgen Reactions on White Rats*

A Preliminary Report

By Tom D. Rowe†

So far as is known, there is no proven curative treatment for third-degree roentgen reactions. In March 1935, Drs. C. E. and Creston Collins called attention to an apparently successful treatment of X-ray reactions by the use of fresh *Alœ vera* leaf (1). Since that time various medical journals have carried articles by physicians reporting the effectiveness of this leaf in treating such burns (2, 3, 4, 5, 6, 7, 8, 9, 10). These reports have been sufficiently promising to indicate that this leaf possesses curative properties. However, all of the work has been done on individual cases, and no reports have been made on experimental work in which controls were used. It appeared, therefore, that before *Alœ vera* could be given full credit for beneficial effects, controlled experimental work should be done.

With this in mind, Dr. C. A. Pohle, professor of radiology, University of Wisconsin, was consulted as to the animals to use and what section of the animals was best suited for the study of irradiation reactions. He suggested the skin of the back of white rats. The first few months of work were entirely experimental, and were directed toward determining what technique and dosage of roentgen rays to use. Twenty-four rats were used for this purpose. At the conclusion of this work, the actual problem was begun.

EXPERIMENTAL

Dr. F. B. Mandeville, professor of radiology at the Medical College of Virginia, coöperated in all X-ray treatments and supervised their administration. In carrying out this problem, the following procedure was employed: First, the rats were anesthetized by intraperitoneal injection of pentobarbital. The hair on the back was then removed with scissors and the rats irradiated individually. During irradiation, each rat was entirely covered with lead foil except for two areas on the back. These areas were 2 cm. square and approximately $2^1/_2$ cm. apart. The rats were given a total of 4000 r., using no filter with 100 kilovolts, 5 milliamperes at a distance of 22.5 cm. They were given the 4000 r. in two doses, 2000 r. one week and 2000 r. a week later. Administration of 4000 r. in a single dose frequently caused death. Using the above factors, 300 r. is the dose which will produce threshold erythema on the average human adult.

Treatment was administered to the rats in groups of eight. Three weeks after the first 2000 r. had been given, each rat showed severe, third-degree X-ray reactions on each of the exposed areas. Photographs were taken at this time and treatment with *Alœ vera* was begun (Fig. 1).

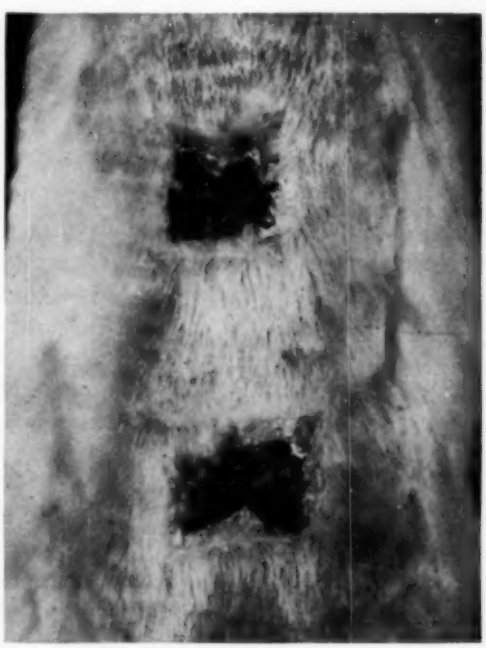

Fig. 1.—Typical Irradiated Areas at Beginning of Treatment.

One irradiated area was used as a control, receiving no treatment other than two applications daily of cotton packs saturated with normal saline

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solution. The other area was treated twice daily with about 1 Gm. of fresh aloe jell, the gelatinous material found inside the leaf. This was applied for four hours at a time and the area was washed with normal saline before applying fresh jell. This procedure was continued for fourteen days on each of the rats in a group. Two days after conclusion of this treatment the animals were photographed, thus giving a series of "before and after" pictures. During treatment, and from four to six weeks afterward, they were kept in separate cages. At the end of that time they were grouped together and reserved for further observation. The jell was kept in place on the rats by means of jackets and collars devised for this purpose. These are shown in Fig. 2.

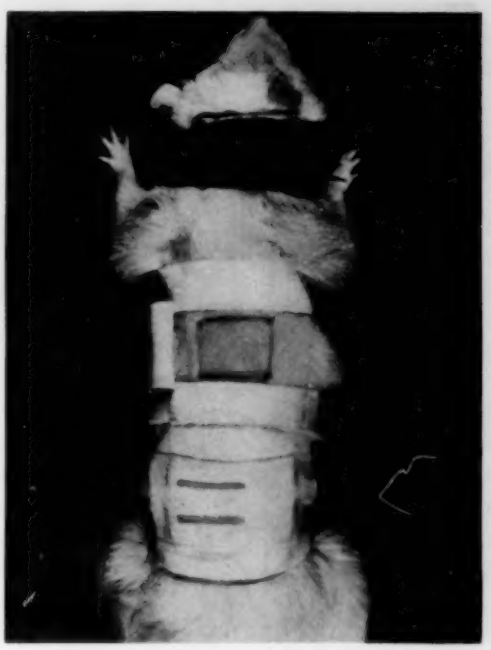

Fig. 2.—Collar and Jacket Used to Apply Jell.

Forty rats were irradiated in the manner described above. Five of these were used as check controls, receiving no jell or saline treatment. Of the 40 rats irradiated, 31 lived until conclusion of the jell treatment. Three of these were in the check-control group. Thus, 28 rats were treated with the jell.

The five groups gave results shown in the following table:

	I	II	III	IV	V
	Improvement[a] with Jell		Both Areas Alike at End of Treatment	Improvement with Saline	
	Definite	Slight		Slight	Definite
Group No. 1 3 rats	2	0	1	0	0
Group No. 2 6 rats	0	1	1	1	3
Group No. 3 8 rats	3	1	4	0	0
Group No. 4 4 rats	2	1	0	0	1
Group No. 5 7 rats	3	1	2	0	1

[a] Improvement based on comparison of the two irradiated areas on the rat.

By combining all of the results given in Columns I and II, it is shown that 14 areas, or 50% of those treated with the jell, showed increased rate of healing. Of these, 10 areas, or 35.72%, showed nearly complete healing at the conclusion of treatment. This number is twice that which showed marked improvement with saline.

The results on all five columns can be interpreted by statistical means. Using +2 for each animal in Column I, +1 for Column II, 0 for Column III, −1 for Column IV and −2 for Column V, it is shown that the probability that there is benefit with the jell is 9/10. This is not considered certainty.[1]

It will be noted that Group No. 2 gave results reverse to the other four groups. There is no explanation for this.

Of the three control animals which lived, two showed no improvement on either area. On the third, both areas were nearly healed two weeks after the last administration of X-ray.

[1] Statistical interpretation done by Mr. J. A. Bradford, Research Laboratory, American Tobacco Company, Richmond, Va.

After conclusion of the jell treatment, the rats were examined once a month. At three and a half months scabs 1 cm. square had appeared on the saline-treated areas of three of the rats. These did not increase in size.

One year after the last group had been treated, 13 of the rats were living. One of these was from the check control group. All the others had been treated with the jell. Of these, both areas showed complete macroscopic healing on nine rats. A small (0.5 x 0.5 cm.) scab was present on the untreated area of one rat, the treated area being completely healed. A pronounced scab ($1^1/_2$ cm. x $1^1/_2$ cm.) was present on the treated area of one rat, the untreated area being completely healed. The three animals which were previously mentioned as showing a recurrence of injury at $3^1/_2$ months were not alive when the examination was made at the end of one year.

When possible, the dead rats were turned over to the pathology department for histopathological ex-

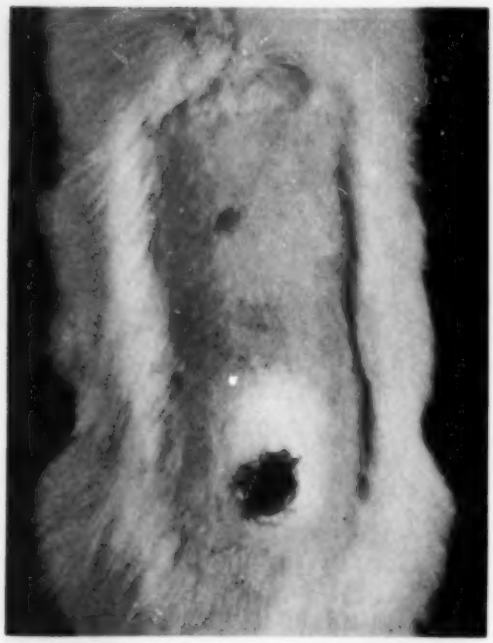

Fig. 3.—Typical Case of Definite Improvement.

aminations. These were done by Dr. George Z. Williams, associate professor of pathology, Medical College of Virginia. He examined 14 rats. Five of these died during different periods of treatment. Nine died at periods ranging from one week to twelve months after treatment. He made the following report on his examinations:

1. Animals which died during treatment: "No qualitative variations could be found to distinguish the treated and untreated irradiated rats."

Fig. 4.—Typical Case of Slight Improvement.

2. Animals which died after treatment: "The healed scars were different histologically only in degree of fibrosis and atrophy of the skin, no qualitative changes being discernible between anterior and posterior areas."

SUMMARY

To date, no definite conclusions have been drawn from the work because: (1) too few animals have received treatment; (2) the 14-day period of treatment is too short a time on which to base final conclusions.

From the results obtained, fresh *Alœ vera* jell shows some promise of being of value in the treatment of X-ray reactions. Plans have been made to continue this problem along the lines already pursued. A larger number of animals is to be treated for a longer period of time.

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Red Squill, VII. Influence of Altitude upon Toxicity to Albino Rats*

By Justus C. Ward, H. J. Spencer, D. Glen Crabtree and F. E. Garlough

Powdered red squill (*Urginea maritima*) is used as a toxic agent for the control of rats in all parts of the country, and since it is employed as a crude powder and the

* From the Control Methods Research Laboratory, Bureau of Biological Survey, U. S. Department of the Interior.

bulbs vary considerably in their content of the rat-killing principle, frequent bioassays should be made in order to produce a reliable product. In comparing the toxicities obtained from bioassays of red squill in our laboratory in Denver, Colorado, with the results of similar tests of the same powder at a lower altitude, the percentages of kill in Denver were consistently higher than those observed at a lower elevation. On analysis of these data it became apparent that altitude is an important factor in the toxic action of red squill.

Changes in the effects of drugs due to altitude have been recognized for well over a century (1). More recently Macht reported (2), in 1931, that digitalis is more toxic to cats at high altitudes in the Blue Ridge Mountains, the Rocky Mountains and in the Tyrolean Alps than at Baltimore. Lehman and Hanzlik (3), in 1932, stated that the emetic and fatal doses of digitalis are 40% to 22% less for pigeons at an altitude of 10,000 feet than at sea level, and these authors also found the same tendencies shown by extremes of fatal doses for a limited number of cats. In 1935 Moore and Ward (4) demonstrated that strychnine is more toxic to tame albino rats and Columbian ground squirrels at an altitude of 5000 feet than at sea level. A. Cannava (5), in 1938, studied the action of chloral on adult rabbits on Mt. Etna (altitude 3000 meters) and found that its action was notably reinforced over the same dosage at sea level. In contrast to these findings, A. Rabbeno (6), in 1937, reported that the slow intravenous infusion of digilanid in guinea pigs at high altitudes resulted in an increase in the M. L. D. of 47% in ten days, the proportion gradually receding in twenty days, however, to 28.7%.

Table I.—Experiments Conducted at an Altitude of Approximately 14,200 Feet

No. and Sex	Weight, Gm.	Dose[a]	Result	No. and Sex	Weight, Gm.	Dose[a]	Result
1. M	121	250	Died	41. F	123	75	Died
2. M	134	250	Died	42. F	131	75	Died
3. M	135	250	Died	43. F	142	75	Died
4. M	143	250	Died	44. F	145	75	Died
5. M	143	250	Died	45. F	147	75	Died
6. M	146	250	Survived	46. F	150	75	Died
7. M	147	250	Survived	47. F	150	75	Died
8. M	153	250	Died	48. F	152	75	Died
9. M	158	250	Died	49. F	152	75	Died
10. M	162	250	Survived	50. F	155	75	Died
11. M	145	200	Died	51. F	112	50	Died
12. M	147	200	Died	52. F	125	50	Survived
13. M	150	200	Survived	53. F	132	50	Died
14. M	152	200	Died	54. F	135	50	Died
15. M	152	200	Survived	55. F	146	50	Died
16. M	153	200	Died	56. F	150	50	Died
17. M	155	200	Died	57. F	155	50	Survived
18. M	163	200	Died	58. F	157	50	Died
19. M	165	200	Died	59. F	164	50	Died
20. M	170	200	Died	60. F	177	50	Died
21. M	128	150	Died	61. F	104	40	Died
22. M	138	150	Died	62. F	126	40	Survived
23. M	140	150	Died	63. F	131	40	Survived
24. M	141	150	Died	64. F	135	40	Died
25. M	141	150	Survived	65. F	144	40	Survived
26. M	144	150	Died	66. F	146	40	Survived
27. M	145	150	Died	67. F	152	40	Died
28. M	148	150	Died	68. F	156	40	Died
29. M	150	150	Died	69 F	160	40	Died
30. M	157	150	Died	70. F	164	40	Died
31. M	165	100	Survived	71. F	113	25	Survived
32. M	168	100	Died	72. F	114	25	Survived
33. M	170	100	Died	73. F	115	25	Survived
34. M	173	100	Died	74. F	115	25	Survived
35. M	177	100	Survived	75. F	122	25	Survived
36. M	183	100	Died	76. F	125	25	Survived
37. M	185	100	Survived	77. F	126	25	Survived
38. M	200	100	Survived	77. F	133	25	Died
39. M	213	100	Survived	79. F	142	25	Survived
40. M	215	100	Survived	80. F	164	25	Survived

[a] Milligrams of red squill powder per kilogram of body weight.
[b] More than 80% of the animals listed above this line died, while less than 50% of those listed below this line succumbed.

Table II.—Experiments Conducted at an Altitude of 717 Feet

No. and Sex	Weight, Gm.	Dose[a]	Result	No. and Sex	Weight, Gm.	Dose[a]	Result
81. M	206	1250	Died	131. F	132	800	Died
82. M	210	1000	Died	132. F	166	700	Died
83. M	223	750	Died	133. F	145	600	Died
84. M	214	750	Died	134. F	165	500	Died
85. M	229	717	Died	135. F	167	500	Died
86. M	230	695	Died	136. F	155	400	Died
87. M	219	610	Died	137. F	153	400	Died
88. M	238	500	Died	138. F	158	300	Died
89. M	221	500	Died	139. F	172	200	Died
90. M	233	250	Survived	140. F	160	100	Died
91. M	169	700	Died	141. F	131	100	Survived
92. M	173	700	Died	142. F	140	100	Survived
93. M	182	700	Died	143. F	146	100	Died
94. M	189	700	Died	144. F	158	100	Died
95. M	204	700	Died	145. F	161	100	Died
96. M	205	700	Died	146. F	165	100	Died
97. M	213	700	Died	147. F	165	100	Died
98. M	214	700	Died	148. F	172	100	Died
99. M	226	700	Died	149. F	173	100	Died
100. M	228	700	Died	150. F	176	100	Died
101. M	134	600	Died	151. F	110	50	Died
102. M	182	600	Survived	152. F	146	50	Died
103. M	183	600	Died	153. F	152	50	Survived
104. M	187	600	Died	154. F	153	50	Survived
105. M	192	600	Died	155. F	163	50	Died
106. M	193	600	Died	156. F	164	50	Died
107. M	207	600	Died	157. F	165	50	Died
108. M	219	600	Died	158. F	167	50	Survived
109. M	220	600	Survived	159. F	171	50	Survived
110. M	224	600	Died	160. F	172	50	Died
111. M	162	400	Died				
112. M	177	400	Survived				
113. M	177	400	Survived				
114. M	188	400	Died				
115. M	190	400	Died				
116. M	195	400	Survived				
117. M	210	400	Survived				
118. M	215	400	Died				
119. M	221	400	Survived				
120. M	248	400	Survived				
121. M	159	250	Survived	161. F	126	25	Survived
122. M	162	250	Survived	162. F	170	25	Survived
123. M	177	250	Survived	163. F	170	25	Survived
124. M	179	250	Survived	164. F	172	25	Survived
125. M	203	250	Survived	165. F	183	25	Survived
126. M	205	250	Survived	166. F	186	25	Survived
127. M	207	250	Survived	167. F	187	25	Survived
128. M	213	250	Survived	168. F	193	25	Survived
129. M	216	250	Survived	169. F	194	25	Survived
130. M	216	250	Survived	170. F	201	25	Survived

[a] Milligrams of red squill powder per kilogram of body weight.
[b] More than 80% of the animals listed above this line died, while less than 50% of those listed below this line succumbed.

Table III.—Comparison of Results

Location and Altitude, Feet	Males				Females			
	Dosage[a] Range	Percentage of Kill	Dosage[a] Range	Percentage of Kill	Dosage[a] Range	Percentage of Kill	Dosage[a] Range	Percentage of Kill
Mt. Evans, Colo., 14,200	150–200	80	100	40	40–75	80	25	10
Denver, Colo., 5280	250	87	200	47	75	87	50	47
Winchester, Va.,	600–700	90	250–400	20	100	80	25–50	30

[a] Milligrams of red squill powder per kilogram of body weight.

In order to study the influence of altitude on the toxic action of red squill, toxicity determinations were carried out at the Cosmic Ray Laboratory of the University of Denver, located on top of Mt. Evans, Colorado, at an elevation of approximately 14,200 feet; at Denver, Colorado, elevation 5280 feet; and at Winchester, Virginia, altitude 717 feet.

EXPERIMENTAL

Rats used in these experiments were from Denver stock and were kept upon the same diet of fox chow

throughout this entire study. The rats from the Denver colony were taken to the Mt. Evans laboratory and allowed to remain at that altitude six days before being used for toxicity determinations. Rats, with an adequate supply of food, were shipped from Denver to Winchester, Virginia, and were allowed thirty days for acclimatization before being used for toxicity studies. The red squill used in these determinations was a standardized powder prepared in the Denver laboratory from a single bulb of high potency. The experiments were carried out using the same technique as reported in a previous paper (7). Data obtained at Mt. Evans and at Winchester, Virginia, were compared with those obtained in the Denver laboratory on the same red squill preparation. The results of the Denver experiments were previously reported in the study of sex factors in the toxicity of red squill (7).

DISCUSSION

The quantities of red squill fed were chosen in such a manner that 75% of the doses given were in the range in which from 80% to 90% of the animals were killed and 25% in the range in which most of the animals survived. These ranges were determined by preliminary experiments in which graduated doses were used. The latter series, one for each sex, are reported in Table II (males, numbers 81–90; females, 131–140, inclusive). In all cases the animals were fasted twenty-four hours before the experiments. The comparison of results given in Table III clearly shows that white rats taken from the same Denver colony to the top of Mt. Evans (altitude 14,200 feet) and to Winchester, Virginia (altitude 717 feet) varied decidedly in their resistance to red squill powder. The findings at Denver, Colorado (altitude 5280 feet), were intermediate between those obtained at the extremes of altitude, as is demonstrated by the fact that 80–90% of the male rats were killed at a dosage of 150–200 mg./Kg. on Mt. Evans, at 250 mg./Kg. in Denver and at 600–700 mg./Kg. in Winchester. Also, in the lower concentrations at which the red squill powder was used, 40% of the male rats were killed at 100 mg./Kg. on Mt. Evans, 47% at 200 mg./Kg. in Denver and only 20% at 250–400 mg./Kg in Winchester. These results indicate that male rats are approximately three times as resistant to red squill at an altitude of 717 feet as they are at 14,200 feet, and about twice as resistant at 717 feet as at 5280 feet. The experiments on female rats at the three elevations demonstrated tendencies in the same direction, but the differences were much less marked.

CONCLUSIONS

1. Altitude is an important factor in the toxicity of powdered red squill to white rats.
2. The influence of altitude is shown primarily on the toxicity to the male rats.
3. The susceptibility of females is little changed by wide variations in altitude.
4. Considerable differences in altitude must be considered in the interpretation of bioassays of red squill powder.

ACKNOWLEDGMENTS

The high altitude studies were made possible through permission given us by Dr. Joyce C. Stearns, in charge of the Cosmic Ray Laboratory of the University of Denver, located 65 miles west of Denver, Colorado, on the top of Mt. Evans, to use the facilities of this laboratory.

We also appreciate the six-day animal care given our experimental rats by Arnold Mathees and Sherman Sundet, Cosmic Ray Laboratory attendants.

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Red Squill, VIII. Further Notes on Bioassay Methods*

By Justus C. Ward, D. Glen Crabtree and F. E. Garlough

For some years red squill has been used in the United States in rat-control projects. As it is a crude plant product, it is subject to the variations inherent in such a material. Many workers have recognized the fact that red squill will differ from time to time and from sample to sample in its rat-killing ability. This uncertainty in squill action has led to several attempts to introduce adequate bioassays at many separate laboratories in the country. Some years ago comparative tests were run with the same red squill powders at three laboratories and significant differences in results were obtained. These were discussed in a paper by J. C. Munch, *et al.* (1). For obvious reasons a series of studies has been carried out to determine the influence of various controllable factors on the toxicity of red squill. The first paper detailing some results of these experiments (2) showed that male rats are more than twice as resistant to this poison as are female rats and proved that this is due to the presence of the male hormone, testosterone. The second (3), which has been submitted for publication, shows the effect of altitude on the toxicity of the red squill powder, proving that there is a direct relationship between the two—the higher the altitude, the higher the toxicity.

Table I.—Influence of the Method of Preparing Bioassay Bait on Red Squill Toxicity

Test *A*. The red squill powder was weighed for each rat individually and then mixed with bait in quantity sufficient to make the total food offered each rat just 1 per cent of its body weight.

Rat No.[a]	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result	Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result
1	M	193	1,000	Died—O. N.[b]	11	F	139	500	Died—O. N.[b]
2	M	205	750	Died—O. N.[b]	11	F	147	500	Died—O. N.[b]
3	M	212	500	Died—O. N.[b]	13	F	152	400	Died—O. N.[b]
4	M	224	500	Died—O. N.[b]	14	F	161	400	Died—O. N.[b]
5	M	224	400	Died—O. N.[b]	15	F	169	250	Died—O. N.[b]
6	M	263	400	Died—3 days	16	F	178	250	Died—O. N.[b]
7	M	269	250	Survived	17	F	178	200	Died—O. N.[b]
8	M	270	250	Survived	18	F	179	150	Died—O. N.[b]
9	M	273	200	Died—2 days	19	F	181	100	Died—4 days
10	M	279	150	Survived	20	F	184	50	Survived

Test *B*. The red squill powder and the bait were both weighed individually, but only enough bait was added to make the powder amount to 10 per cent of the total food given.

No males were used in this series.

Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result
21	F	135	500	Died—O. N.[b]
22	F	154	500	Died—O. N.[b]
23	F	155	400	Died—O. N.[b]
24	F	161	400	Died—O. N.[b]
25	F	162	250	Died—O. N.[b]
26	F	171	250	Died—O. N.[b]
27	F	182	200	Died—O. N.[b]
28	F	183	150	Died—O. N.[b]
29	F	189	100	Died—O. N.[b]
30	F	205	50	Died—O. N.[b]

Test *C*. The red squill powder and the bait were first blended into a carefully stirred bioassay mixture containing exactly 10 per cent squill powder. This food was then weighed out in the proper proportions to give the desired dose of poison per rat.

Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.	Result	Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result
31	M	158	1,000	Died—O. N.[b]	41	F	126	500	Died—O. N.[b]
32	M	166	750	Died—O. N.[b]	42	F	152	500	Died—O. N.[b]
33	M	193	500	Died—O. N.[b]	43	F	154	400	Died—O. N.[b]
34	M	193	500	Died—O. N.[b]	44	F	155	400	Died—O. N.[b]
35	M	196	400	Died—O. N.[b]	45	F	173	250	Died—O. N.[b]
36	M	202	400	Died—O. N.[b]	46	F	174	250	Died—O. N.[b]
37	M	207	250	Died—O. N.[b]	47	F	175	200	Died—O. N.[b]
38	M	208	250	Died—2 days	48	F	176	150	Died—O. N.[b]
39	M	216	200	Survived	49	F	177	100	Died—O. N.[b]
40	M	220	100	Survived	50	F	199	50	Died—5 days

[a] Milligrams of red squill powder per kilogram of body weight.
[b] Over night.

* From the Control Methods Research Laboratory, U. S. Biological Survey, United States Department of the Interior, Denver, Colorado.

The present paper shows the effects on the toxicity of red squill of (*a*) the method of preparing the bioassay bait; (*b*) the concentration of red squill powder in the bioassay bait; and (*c*) the strain of rat used in the bioassay.

EXPERIMENTAL

In the tests listed in Table I the powder used was made from a single bulb of high potency; in those in Table II, a fortified powder having three times the toxicity of the original crude drug, the powder having been prepared in the Control Methods Research Laboratory; and in those in Table III, six red squill powders of different degrees of toxicity. The tables are self-explanatory.

In all the studies a standard food mixture was used consisting of 85 parts of dried and screened bread crumbs and 5 parts of mineral oil. The bioassay baits were prepared immediately before being fed to the rats. The rats were maintained on

Table II.—Effect of the Percentage of Red Squill Powder in the Bait on Its Toxicity

Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result	Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result
		10 Per Cent Bait					8 Per Cent Bait		
51	M	127	500	Died—2 days	74	M	123	500	Died—3 days
52	M	101	500	Died—2 days	75	M	108	500	Died—2 days
53	M	102	500	Died—2 days	76	M	109	500	Survived
54	M	103	500	Died—2 days	77	M	109	500	Died—O. N.[b]
55	M	104	500	Died—O. N.[b]	78	M	110	500	Died—5 days
56	M	105	500	Died—3 days	79	M	115	500	Died—2 days
57	M	106	500	Died—2 days	80	M	116	500	Died—1 day
58	M	134	300	Died—6 days	81	M	128	300	Died—2 days
59	M	185	300	Died—2 days	82	M	177	300	Died—2 days
60	M	186	300	Died—4 days	83	M	178	300	Died—4 days
61	M	189	300	Died—3 days	84	M	179	300	Died—6 days
62	M	190	300	Died—O. N.	85	M	181	300	Died—O. N.
63	M	191	300	Died—4 days	86	M	181	300	Survived
64	M	195	300	Died—O. N.	87	M	184	300	Survived
65	M	137	200	Survived	88	M	132	200	Survived
66	M	138	200	Survived	89	M	133	200	Survived
67	M	140	200	Died—2 days	90	M	135	200	Survived
68	M	80	200	Died—O. N.	91	M	82	200	Died—O. N.
69	M	89	200	Died—5 days	92	M	90	200	Died—4 days
70	M	101	200	Died—3 days	93	M	98	200	Died—5 days
71	M	103	200	Died—8 days	94	M	105	200	Died—5 days
72	M	110	200	Survived	95	M	109	200	Died—5 days
73	M	113	200	Survived	96	M	113	200	Died—2 days
		5 Per Cent Bait					3 Per Cent Bait		
97	M	110	500	Died—2 days	120	M	101	500	Survived
98	M	116	500	Died—2 days	121	M	124	500	Survived
99	M	117	500	Died—O. N.[b]	122	M	124	500	Died—O. N.[b]
100	M	118	500	Died—O. N.[b]	123	M	126	500	Died—1 day
101	M	120	500	Survived	124	M	126	500	Survived
102	M	121	500	Died—3 days	125	M	124	500	Died—2 days
103	M	123	500	Died—1 day	126	M	129	500	Died—O. N.
104	M	114	300	Died—2 days	127	M	104	300	Survived
105	M	169	300	Died—2 days	128	M	141	300	Died—2 days
106	M	170	300	Died—2 days	129	M	161	300	Survived
107	M	175	300	Died—2 days	130	M	166	300	Survived
108	M	176	300	Died—3 days	131	M	166	300	Died—3 days
109	M	176	300	Died—1 day	132	M	167	300	Died—2 days
110	M	177	300	Survived	133	M	168	300	Died—2 days
111	M	133	200	Survived	134	M	116	200	Survived
112	M	135	200	Survived	135	M	119	200	Survived
113	M	136	200	Survived	136	M	127	200	Survived
114	M	86	200	Died—7 days	137	M	89	200	Died—5 days
115	M	93	200	Died—5 days	138	M	95	200	Died—5 days
116	M	97	200	Died—4 days	139	M	96	200	Died—1 day
117	M	106	200	Survived	140	M	107	200	Survived
118	M	109	200	Died—7 days	141	M	108	200	Died—5 days
119	M	116	200	Died—2 days	142	M	130	200	Died—5 days

[a] Milligrams of red squill powder per kilogram of body weight.
[b] Over night.

This table shows the following percentage of kills:

Dose	10	8	5	3
500 mg./Kg.	100	85.7	85.7	57.2
300 mg./Kg.	100	71.5	85.7	42.9
200 mg./Kg.	55.5	66.7	55.5	55.5

These figures indicate that the concentration of the poison in the bioassay bait is of decided importance.

a balanced diet in the laboratory for at least 10 days before being used for the experiments. On the evening of the day before the tests were made the food was removed from the stock cages, and the following morning the test animals were removed and weighed into individual feeding cages. The proper doses were computed and the baits weighed. The rats were fed between 3 P.M. and 6 P.M., that is, after a 24-hour fasting period. Water was available in adequate quantity at all times.

Table I shows that, in so far as the two 10 per cent bait methods are concerned, there is no discernible difference, but that when the same doses are given in a bait carrying 1 per cent body weight concentration there is evidence, when the smaller doses are compared, that the larger quantity of food interfered with the action of the squill. Since the Test *C* method of bioassay bait preparation will save almost half of the weighings involved in the usual feeding test, this laboratory has adopted it as our standard system.

Table III shows that the three strains of rats studied did not exhibit any detectable difference in susceptibility to six different red squill samples of varying toxicities. The rats used were of approximately the same ages, the difference in weights being due to the more rapid growth characteristic of the Yale strain. The Baxter strain

Table III.—Influence of the Strain of Rat on Red Squill Toxicity

A. Special bulb 10 per cent bait

Baxter Strain				Denver University Strain				Yale Strain			
Rat No.	Wt., Gm.	Dose, mg./Kg.[a]	Result	Rat No.	Wt., Gm.	Dose, mg./Kg.	Result	Rat No.	Wt., Gm.	Dose, mg./Kg.[a]	Result
143	105	300	Died—O. N.[b]	155	117	300	Died—4 days	167	131	300	Died—O. N.[b]
144	142	300	Died—O. N.[b]	156	142	300	Died—3 days	168	217	300	Died—3 days
145	143	250	Died—3 days	157	153	250	Died—6 days	169	220	250	Survived
146	170	250	Died—3 days	158	161	250	Died—6 days	170	240	250	Died—O. N.
147	171	250	Survived	159	165	250	Died—O. N.[b]	171	242	250	Died—5 day[b]
148	265	250	Died—2 days	160	179	250	Died—O. N.[b]	172	293	250	Died—O. N.s
149	124	250	Died—O. N.[b]	161	135	250	Died—O. N.[b]	173	195	250	Died—O. N.[b]
150	140	250	Died—O. N.[b]	162	144	250	Died—O. N.[b]	174	215	250	Died—4 day[b]
151	144	250	Died—O. N.[b]	163	151	250	Survived	175	222	250	Died—4 days
152	169	250	Died—2 days	164	160	250	Died—O. N.[b]	176	238	250	Died—O. N.s
153	170	200	Died—3 days	165	168	200	Died—3 days	177	247	200	Died—6 day[b]
154	214	200	Died—3 days	166	176	200	Died—O. N.[b]	178	292	200	Died—6 days

B. "Caked" squill No. 1705 10 per cent bait

Baxter Strain				Yale Strain			
Rat No.	Wt., Gm.	Dose, mg./Kg.	Result	Rat No.	Wt., Gm.	Dose, mg./Kg.	Result
179	143	2500	Died—1 day	184	230	2500	Died—1 day
180	164	2000	Died—2 days	185	246	2000	Died—O. N.[b]
181	183	1500	Died—1 day	186	250	1500	Died—1 day
182	208	1000	Died—6 days	187	276	1000	Survived
183	216	750	Survived	188	287	750	Died—O. N.[b]

C. Red squill powder No. 1706 10 per cent bait

189	148	2500	Died—O. N.[b]	194	235	2500	Died—O. N.[b]
190	161	2000	Died—2 days	195	245	2000	Died—O. N.[b]
191	184	1500	Died—1 day	196	260	1500	Died—O. N.[b]
192	209	1000	Died—1 day	197	271	1000	Died—2 days
193	215	750	Died—3 days	198	284	750	Died—O. N.[b]

D. Red squill powder No. 1699 (Fortified 2×) 10 per cent bait

199	120	600	Died—O. N.[b]	205	215	600	Died—O. N.[b]
200	167	500	Died—1 day	206	250	500	Died—O. N.[b]
201	179	400	Died—O. N.[b]	207	257	400	Died—O. N.[b]
202	192	300	Died—O. N.[b]	208	278	300	Died—O. N.[b]
203	199	250	Died—O. N.[b]	209	281	250	Died—O. N.[b]
204	225	200	Died—3 days	210	310	200	Died—2 days

E. Red squill powder No. 1699 (Fortified 7×) 5 per cent bait

211	165	250	Died—O. N.[b]	217	211	250	Died—O. N.[b]
212	170	200	Died—2 days	218	251	200	Died—1 day
213	172	150	Survived	219	254	150	Died—1 day
214	196	100	Survived	220	278	100	Survived
215	197	75	Survived	221	280	75	Survived
216	236	50	Survived	222	354	50	Survived

F. Red squill powder No. 1709 10 per cent bait.

223	142	2500	Died—O. N.[b]	229	229	2500	Died—2 days
224	165	2000	Died—3 days	230	247	2000	Died—6 days
225	183	1500	Died—O. N.[b]	231	257	1500	Died—O. N.[b]
226	191	1250	Died—2 days	232	277	1250	Died—O. N.[b]
227	206	1000	Died—2 days	233	285	1000	Died—2 days
228	217	750	Died—6 days	234	305	750	Died—6 days

[a] Milligrams of red squill powder per kilogram of body weight.
[b] Over night.

of rats is a hybrid one, predominately Wistar. The Denver University strain is a hybrid which has been inbred at the local colony for many years. The Yale strain used was a pure line obtained from the University of Colorado School of Medicine.

CONCLUSIONS

1. Mixing weighed quantities of red squill powder with food to make 1 per cent body weight bioassay baits is not satisfactory for accurate studies, since the ratio of poison to food will vary, and a definite food interference is noted when small doses of squill are fed.

2. It is satisfactory either to weigh out both the red squill powder and the bait for the bioassay bait and mix for each individual test animal, or previously to blend a standard concentration bait and weigh the proper quantities of this food to give the doses desired.

3. The percentage of red squill powder in the bioassay bait is of importance, both as regards the ultimate toxicity and the speed of action of the poison.

4. The strain of rat used does not affect the bioassay, when the animals have been maintained on the same diet for at least a week before the beginning of the bioassay. The rats used should be of approximately the same age when bioassays on two or more strains are to be carefully compared.

5. Depending upon the quality of the squill powder being tested, from 0 to 33⅓ per cent of the animals survive longer than three days, although they ultimately die with typical red squill symptoms, and accordingly must be counted in the bioassay. In many cases the tests must be allowed to continue for seven or eight days.

6. Female rats are more than twice as susceptible to red squill powder as are male rats.

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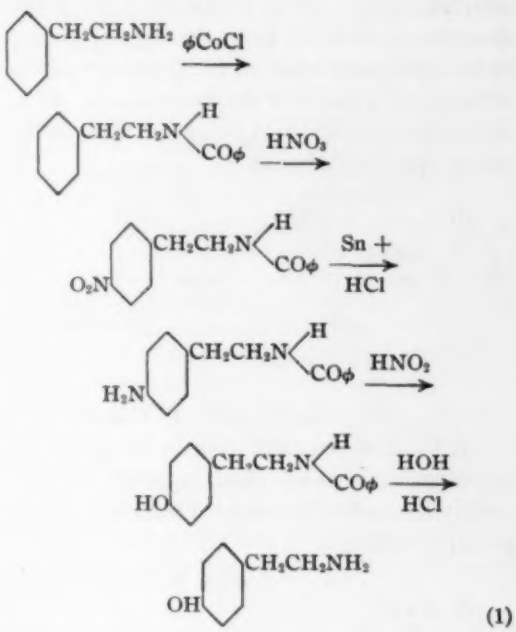
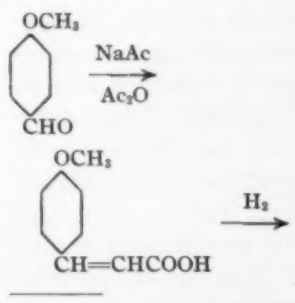
The Synthesis of Hydroxymandelonitrile Dibenzoates

By Kenneth E. Hamlin, Jr., and Walter H. Hartung*

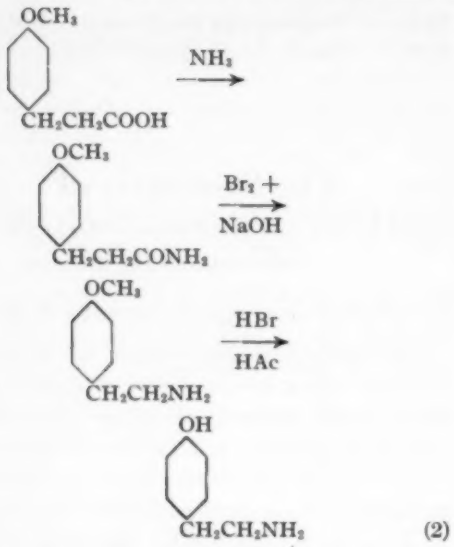
The biological importance of tyramine and the rather incompletely reported pharmacological studies of other β-hydroxyphenylethylamines led to the investigation of possible intermediates for a synthesis of tyramine and its isomers. Previous syntheses have been reported by Barger and Walpole (1) from phenylethylamine and from anisaldehyde as indicated by:



(1)

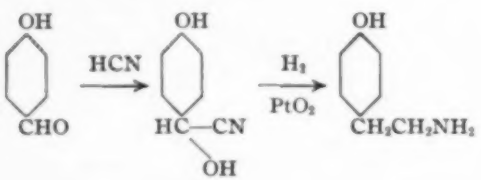


* University of Maryland Alumni Fellow, 1938–1939; Wm. R. Warner Fellow 1939–1940.

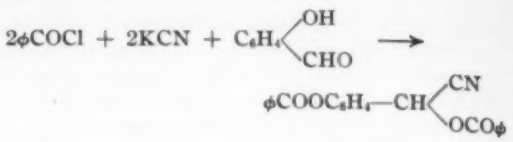


(2)

Buck (2) reports a new synthesis of tyramine through the reduction of hydroxymandelonitriles. His preparation of *p*-hydroxymandelonitrile from the corresponding hydroxybenzaldehyde in 38% yields and its subsequent reduction to tyramine in 48% yields (the overall yield being less than 20%) can be represented thus:

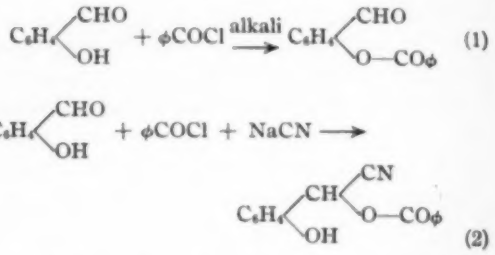


It has been established by Francis and Davis (3) that benzaldehyde in the presence of potassium cyanide and benzoyl chloride readily forms the benzoate of mandelonitrile, $\phi\text{—CH(O—CO}\phi\text{)—CN}$, ($\phi$ = C_6H_5) in good yields. Hartung (4) has described the catalytic reduction of mandelonitrile to β-phenylethylamine. A combination of these reactions suggested a synthesis of tyramine and its isomers. The hydroxybenzaldehydes, however, because of their phenolic character and the basic nature of the alkali cyanides, simultaneously undergo the Schotten-Bauman reaction, thereby complicating results. Aloy and Rabaut (5) described the preparation of *p*- and *o*-hydroxymandelonitrile dibenzoates in the following way:

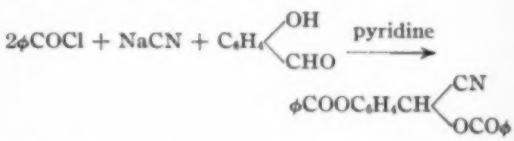


They reported no yields and only the para-compound was analyzed. The ortho- isomer was described as a liquid; no reference was made to the *m*-hydroxymandelonitrile dibenzoate.

Since here are two competing reactions, *viz.*,



it is obvious that the ultimate results will be influenced by the relative speeds of these reactions. Consequently if one can first be completed, the second ought to proceed with a minimum of interference. For example, the esterification of the phenolic hydroxyl may be carried out with benzoyl chloride using a tertiary organic base such as pyridine, and then the cyanhydrin formation with accompanying esterification takes place without interference. Thus, the benzoates of the hydroxybenzaldehydes give quite as satisfactory yields of the cyanhydrin benzoate as does benzaldehyde itself. In practice, however, it is unnecessary first to isolate the ester of the phenolic aldehyde, for it is possible to esterify the hydroxyl group and simultaneously obtain the benzoyl derivative of the cyanhydrin as indicated by:



Thus far, it has been possible to obtain yields up to 67% of *p*-hydroxymandelonitrile dibenzoate by this reaction on *p*-hydroxybenzaldehyde and 45% of *o*-hydroxymandelonitrile dibenzoate from *o*-hydroxybenzaldehyde. Because of the scarcity of *m*-hydroxybenzaldehyde, it has been impossible

to obtain the optimum conditions for the best yields of the *m*-hydroxymandelonitrile dibenzoate. Contrary to the work of Aloy and Rabaut, the *o*-hydroxymandelonitrile dibenzoate was purified as a crystalline solid.

Since these derivatives, which are all stable, crystalline solids, melting without decomposition, may be synthesized with comparative ease, the course of their reduction merits attention. In the light of the previous work, catalytic reduction is expected to proceed according to the following equation:

$$\phi COOC_6H_4CH\begin{matrix}CN\\OCO\phi\end{matrix} \xrightarrow[\text{simultaneous alcoholysis}]{H_2\,(Pd)} HO\cdot C_6H_4\cdot CH_2CH_2NH_2 + 2\phi COOC_2H_5$$

Furthermore, if the reduction can be influenced to take the following course,

$$\phi COOC_6H_4CH\begin{matrix}CN\\OCO\phi\end{matrix} \xrightarrow[\text{simultaneous alcoholysis}]{H_2(Pd)} HO\cdot C_6H_4\cdot CH\begin{matrix}CH_2NH_2\\O{-}CO\phi\end{matrix} + \phi COOC_2H_5$$

the product, $HOC_6H_4CH\begin{matrix}CH_2NH_2\\O{-}CO\phi\end{matrix}$, will prove of unusual interest because in this single molecule are combined the optimum structural elements for pressor activity and a potent anesthesiophore group.

EXPERIMENTAL

Hydroxybenzaldehyde Benzoates.—The preparation of the hydroxybenzaldehyde benzoates is carried out using pyridine as the base in the benzoylation reaction. A slight excess of benzoyl chloride is added to molecular quantities of the hydroxybenzaldehyde and pyridine. Considerable heat is generated and reaction is immediate, but usually the mixture if allowed to stand over night gives better yields. The solution is extracted with ether and washed thoroughly with dilute hydrochloric acid to remove the pyridine and with dilute alkali to remove the excess benzoyl chloride. After removal of the ether, the isomeric hydroxybenzaldehyde benzoates are purified as described below.

The *p*-hydroxybenzaldehyde benzoate is a solid and can be recrystallized from alcohol. On precipitation from ether using petroleum ether, white crystals are obtained as long fine needles, melting from 90.0–90.5°.[1] The phenylhydrazone of *p*-hydroxybenzaldehyde benzoate recrystallized from alcohol gives white, fluffy needles, melting at 173–174°.

The *o*-hydroxybenzaldehyde benzoate is a viscous, water-white liquid, distilling from 184–185° at 2-mm. pressure. Vavon (6) has previously reported the boiling point as 207–208° at 13 mm. The phenylhydrazone from alcohol gives small yellow needles melting at 137–138°.

The *m*-hydroxybenzaldehyde benzoate is a white crystalline compound. On recrystallization from alcohol as fine needles, it melts from 48.5–49.0°. Previous reference to this compound could not be found in the literature.

Hydroxymandelonitrile Dibenzoates.—The hydroxymandelonitrile dibenzoates are prepared from the hydroxybenzaldehyde benzoates in the following way. To molecular quantities of the hydroxybenzaldehyde benzoate and pyridine is added a slight excess of sodium cyanide (as a saturated aqueous solution). On shaking and cooling, the corresponding cyanhydrin is formed as a solid compound. Next, an equivalent quantity of benzoyl chloride is added slowly in small portions. The mixture becomes highly colored and much heat is produced, constant cooling and shaking being necessary. After reaction, if dilute hydrochloric acid is added to the mixture, an oil separates out which solidifies on standing. The solid matter is filtered off and recrystallized from a suitable solvent. Some difficulty was experienced in selecting the proper solvent for purification of each solid isomer.

It was found that if the proper hydroxybenzaldehyde is dissolved in two equivalents of pyridine, a slight excess of sodium cyanide (as the saturated aqueous solution) is added and then two equivalents of benzoyl chloride are added slowly, with constant shaking and cooling, the identical hydroxymandelonitrile dibenzoate can be isolated in substantially the same yield. The latter procedure eliminates isolating the intermediate hydroxybenzaldehyde benzoates.

The *p*-hydroxymandelonitrile dibenzoate is a white crystalline compound. When recrystallized from acetone, the crystals are small platelets melting from 144.5–145.5°. It was obtained in yields up to 67%.

Analysis: Theor. for $C_{22}H_{17}O_4N$: 3.92% N.
Found: 4.09% N.

The *o*-hydroxymandelonitrile dibenzoate was obtained in yields up to 45%. It is best recrystallized from *n*-propyl alcohol, giving pale yellow needle crystals melting from 92.0–92.5°.

[1] This melting point does not agree with that reported by Kopp, *Ann. Chem.*, 277 (1893) 350, who gives the melting point of *p*-hydroxybenzaldehyde benzoate as 72°. His method was repeated; a melting point and mixed melting point checked with that obtained above. Obviously there is some error as the melting point for the benzyl ether of *p*-hydroxybenzaldehyde is given as 72° by Worner, *Ber.*, 29, 142.

Analysis: Theor. for $C_{22}H_{15}O_4N$: 3.92% N.
Found: 4.13% N.

The *m*-hydroxymandelonitrile dibenzoate is a white crystalline compound. It was obtained after some difficulty by recrystallization as fine needles from toluene, cooled in a dry ice-alcohol bath. Its melting point is 118.5–119.5°.

Analysis: Theor. for $C_{22}H_{15}O_4N$: 3.92% N.
Found: 3.98% N.

SUMMARY

The synthesis of the dibenzoates of the three isomeric hydroxymandelonitriles has been investigated. They may be obtained:

(1) by treating the benzoate of the hydroxybenzaldehyde with an equivalent of alkali cyanide and benzoyl chloride;

(2) by treating the hydroxybenzaldehyde with an equivalent of pyridine, an equivalent of alkali cyanide and two equivalents of benzoyl chloride.

Of the three isomeric dibenzoates, the *o*- and the *m*- compounds have been, so far as known, prepared for the first time. The benzoate of *m*-hydroxybenzaldehyde is also reported for the first time.

The reduction behavior of these hydroxymandelonitrile dibenzoates will be investigated.

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Christian Eijkman (1858–1930), discoverer of the cause of beriberi, shared the Nobel Prize for medicine in 1929 with Sir Frederick Hopkins for extensive vitamin research. To his satisfaction, the antineuritic vitamin was isolated as a pure crystalline substance by his countrymen, Janses and Donath, in the laboratory in Batavia which had risen out of his own primitive research quarters.

The Analysis of Sodium Acetate

By R. M. Hitchens, G. W. Ashworth* and W. H. Demaree†*

One common method for the determination of acetic acid in acetates has consisted of distillation from a strongly acid solution and titration of the distillate. Hurd and Fiedler (1) have reported encountering considerable difficulty in the assay of sodium acetate by this process. They found it necessary to maintain the volume of liquid being distilled at 200 cc. and to carry out the distillation over a period of ten hours to prevent high results from carryover of phosphoric acid.

These observations seem surprising to us since we have long used this method as a routine assay method for sodium acetate, both alone and in the presence of sodium carbonate and sodium chloride. In view of the troubles which Hurd and Fiedler experienced we are describing below the apparatus, procedure and the results obtained with known amounts of sodium acetate and acetic acid.

EXPERIMENTAL

Apparatus.—The distillation apparatus employed is illustrated in Fig. 1. It consists of a 250-cc., round-bottom flask connected by means of a ground-glass joint to a spray column filled with small glass helices which in turn leads to a condenser. A small reservoir is fitted to the top of the column to allow addition of water to the flask. The receiver is a 250-cc. Volhard absorption flask. This simple, compact still allows rapid distillation with no carryover. It is widely applicable to a variety of quantitative distillations.

Procedure.—Sufficient sample to contain about 0.02 Gm. mol of sodium acetate is added to the flask, along with 50 cc. of water and 15 cc. of 85% phosphoric acid. Twenty cc. of water are added to the receiver to act as a seal. The solution is distilled to a volume of about 20 cc., the flame removed, 20 cc. of water run in through the reservoir and the solution again distilled to 20 cc. For safety a second 20-cc. portion of water is added and distilled as before. The distillate is titrated with carbonate-free 0.5*N* sodium hydroxide solution, using phenolphthalein or thymol blue as indicator. The entire distillation consumes only 20 minutes.

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The whole analysis can be carried out in an elapsed time of 45 minutes.

Results with Known Amounts of Acetic Acid.—An approximately 0.4*N* solution of acetic acid was prepared by diluting glacial acid with distilled water. Exactly 50.00-cc. portions of this solution were added to the distilling flask and to a 250-cc. Erlenmeyer flask. The former were distilled as above and titrated with a given, carbonate-free, 0.5*N* sodium hydroxide solution; the latter were titrated directly. In this way it was possible to detect any errors introduced by the distillation. The results were as follows:

Table I

Sample	Cc. 0.5*N* Sodium Hydroxide
Not distilled	42.83
Not distilled	42.82
Distilled	42.82
Distilled	42.81
Distilled	42.82

Thus, within the limits of the experimental error of reading a burette no loss occurs in the distillation. All distillates were tested for phosphates by acidifying with nitric acid and adding ammonium molybdate reagent. No yellow precipitate was ever obtained, and, in fact, no yellow coloring even observed. This indicates the absence of phosphoric acid carryover.

Standardization against Pure Sodium Acetate.—Three samples of sodium acetate, A. R., were dried thoroughly at 130° C. About 2 Gm. of each were weighed into the distilling flask, care being taken to avoid pickup of water during weighing. The results of these analyses are given in Table II.

Table II.—Assay of Pure Sodium Acetate

Sample	Weight of Sample, Gm.	Cc. 0.5*N* Sodium Hydroxide	Factor for Sodium Hydroxide	Per Cent Acetate
A	1.8824	45.78	1.0020	100.0
B	1.9571	45.59	1.0020	99.9
C	2.4249	58.68	1.0070	100.0

Once again, within 1 part in 1000 or within the experimental error of volumetric analysis, recovery of acetic acid is quantitative.

This method is applicable in the presence of chloride if a slight excess of silver oxide is added to precipitate the chloride as silver chloride. It is likewise applicable in the presence of carbonates if the distillate is subjected to reduced pressure and a slow stream of carbon dioxide free air led through it for a minute or so before titration.

We have found this distillation procedure to be more precise than the conventional U. S. P. XI method which involves ashing to sodium carbonate, leaching and titrating the sodium carbonate produced. It does away with the errors caused by incomplete washing and by occlusion of carbonates by the unburned carbon. It reduces manipulative losses since it involves less handling. It has proved to give more consistently reproducible results in the hands of the average analyst.

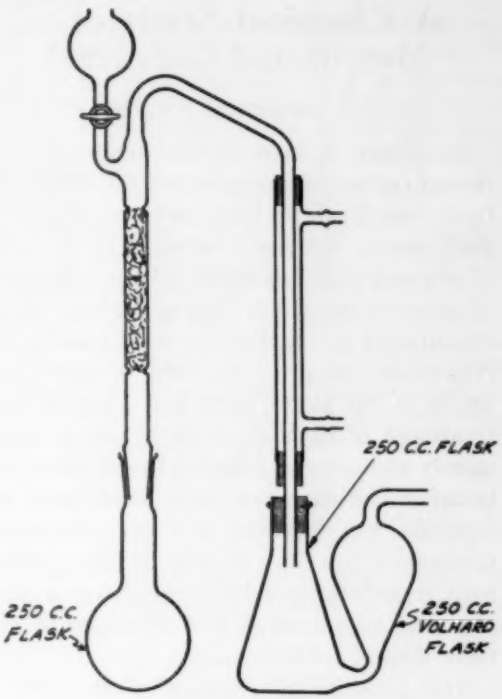

Fig. 1.—Distillation Apparatus.

CONCLUSIONS

A simple, compact, all-glass still which eliminates spray in quantitative distillations is described.

Its use in the quantitative determination of sodium acetate by adding phosphoric acid, distilling and titrating the acetic acid is described.

The method can be adapted to eliminate the effect of chlorides and carbonates.

The errors observed by Hurd and Fiedler are the result of faulty apparatus.

The method has certain definite advantages, notably speed and precision, over the U. S. P. XI ashing procedure.

REFERENCE

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"It is provided in the nature of things that from any fruition of success shall come forth something that shall make a greater effort necessary."—WALT WHITMAN.

A Chemical Study of a Mercurated Carvacrol

By J. B. Abcede and A. C. Santos**

In a desire to increase the therapeutic action of certain organic compounds, attempts have been made to introduce mercury into their nuclei, thereby creating a fertile field of research that has attracted the attention of many investigators who, as a result, have contributed to chemistry a good number of important organic mercury compounds. Many of the latter have found use in the treatment of diseases, while others present merely an interesting study of their chemical behavior. Because of their importance in medicine, the chemistry of organic mercury compounds has been studied to throw some light regarding the relationship between the chemical constitution and therapeutic effects of these substances.

The mercury derivatives of thymol, a phenol for instance, have been the subject of investigations. The mercury is introduced into the ring, according to Rupp (1), by reacting thymol with mercuric acetate. By nitrating the diacetoxymercuri-carvacrol, the positions occupied by the mercury complex groups have been determined. The nitrated product was found to be identical with the reported 2:6 dinitro-thymol, m. p. 55° C. From the diacetoxymercurithymol other mercury derivatives of thymol were formed.

Following Burt's (2) method of mercuration, carvacrol and mercuric acetate in equimolecular proportion produced a diacetoxymercuri-carvacrol. When one-fifteenth of a mole of carvacrol was made to react with two-fifteenths of a mole of mercuric acetate, a greater percentage of the yield of diacetoxymercuri-carvacrol was obtained.

The carvacrol used in the experiment was obtained from Dr. Fränkel and Dr. Landau in Berlin and was purified by fractional distillation. It was identified by the phenyl urethane of carvacrol, m. p. 136–137°.

It would appear that the best possible positions for anchorage of the acetoxymercuri radicals are the positions *ortho* and *para* to the hydroxyl group in carvacrol. We are at present engaged in the nitration of the mercurated carvacrol to determine the positions occupied by the mercury complex groups. The nitro compound of carvacrol has not yet been obtained in pure form, however.

From the results of our experiments, the mercury in the mercury derivatives of carvacrol seems to be in non-ionic form. These derivatives dissolve in sodium hydroxide solution without the precipitation of mercuric oxide. Hydrogen sulfide does not readily produce a blackening of the diacetoxymercuri-carvacrol held in aqueous suspension. Strong acids, like hydrochloric acid, cause a breakage of the mercury from the organic linking when the substance is heated. The liberated carvacrol becomes manifest by its characteristic odor. A saturated solution of sodium chloride precipitates a dichlormercuri-carvacrol from an alcoholic solution of diacetoxymercuri-carvacrol. From a warm sodium hydroxide solution of diacetoxymercuri-carvacrol a sodium compound of dihydroxymercuri-carvacrol crystallizes out on cooling. It seems that, due to its decreased solubility in alkali, the oxide of dihydroxymercuri-carvacrol with one molecule of water is precipitated as amorphous powder by passing a current of carbon dioxide into a sodium hydroxide solution of diacetoxymercuri-carvacrol. It appears that one of the two mercury atoms becomes linked with the oxygen of the phenolic hydroxyl group while the other is still attached to the ring and to the hydroxyl radical. The linking can possibly take place only with a hydroxymercuri group adjacent to the phenolic hydroxyl radical. Other derivatives of mercurated carvacrol are still under study.

The analyses of these compounds for the percentages of carbon and hydrogen were conducted according to the method of Pregl (3) in the micro-combustion apparatus. The mercury determinations were performed according to the methods of Rupp (1) and the U. S. P. XI.

A dichlormercuri-carvacrol has been reported by Burt (4) to be giving good results

* The College of Pharmacy, University of the Philippines.

in the treatment of various skin diseases. Ointments of dichlormercuri-carvacrol in 2.5% and 5% strengths have been prepared in our laboratories and are being tried in treating cases of athlete's foot, ringworm, eczema and other skin diseases to determine the degree of success with which it can be used as a remedy for skin infections. Burt states that even a 10% ointment of the compound produces no untoward effects.

The 2.5% ointment has been tried in certain cases of burns and it was observed that there was almost instantaneous relief from the attendant pain and that the threatening blisters did not form.

EXPERIMENTAL

Diacetoxymercuri-carvacrol.—In the presence of 12 cc. of glacial acetic acid, 10 Gm. of carvacrol with 42.4 Gm. of mercuric acetate dissolved in 125 cc. of alcohol were refluxed on a water-bath for two and a half hours. The white crystals of diacetoxymercuri-carvacrol that appeared on cooling were filtered off by suction, washed with cold alcohol and water and then dried. The product weighed 29.9 Gm. corresponding to 67.2% of the theoretical yield. It melted at 192–195° C. with decomposition and evolution of gases. Analysis of the substance gave the following results:

5.380 mg. subs.: 4.964 mg. CO_2 and 1.254 mg. H_2O.
0.3030 Gm. subs.: 0.2112 Gm. HgS.

			C	H	Hg
Calcd.:	$C_{14}H_{18}O_5Hg_2$	(667.2)	25.19	2.7	60.12
	$C_{12}H_{16}O_2Hg$	(408.6)	35.24	3.9	49.09
Found:			25.12	2.6	60.11

The white crystalline product obtained by using equimolecular quantities of carvacrol and mercuric acetate and following the same method of preparation melted also with decomposition and evolution of gases at 192–195° C. and gave the following results on analysis:

4.880 mg. subs.: 4.559 mg. CO_2 and 1.160 mg. H_2O.
0.2372 mg. subs.: 12.6 cc. 0.1119*N* NH_4CNS.

		C	H	Hg
Calcd.:	$C_{14}H_{18}O_5Hg_2$	25.19	2.7	60.12
	$C_{12}H_{16}O_2Hg$	35.24	3.9	49.09
Found:		25.16	2.66	59.65

Dichlormercuri-carvacrol.—Ten grams of carvacrol with 42.4 Gm. of mercuric acetate in 200 cc. alcohol and 12 cc. acetic acid were refluxed on a water-bath for three hours and the reaction mixture was filtered while hot into a saturated solution of sodium chloride producing a fine white precipitate. This was filtered with suction, washed with alcohol and water and dried. The yield of 34.22 Gm. was equivalent to 82.7% of the theoretical yield. It occurs as white powder with the property of clinging tenaciously to any surface it comes in contact with. It crystallizes out in needles when dried over sulfuric acid. It is decomposed without fusion at 216–218° C.

5.771 mg. subs.: 4.058 mg. CO_2 and 1.074 mg. H_2O.

		C	H
Calcd.:	$C_{14}H_{12}OHg_2Cl_2$	19.35	2.08
Found:		19.18	2.08

Sodium Compound of Dihydroxymercuri-carvacrol.—Two grams of diacetoxymercuri-carvacrol were dissolved in 10 cc. of 10% sodium hydroxide solution. The mixture is taken up with 10 cc. of boiling water and then filtered. On cooling possibly a sodium compound of dihydroxymercuri-carvacrol crystallizes out. Only a small amount was isolated and it decomposed at 180° C. without fusion. It is insoluble in alcohol and other organic solvents, but dissolves readily in warm water.

Oxide of Dihydroxymercuri-carvacrol.—Two grams of diacetoxymercuri-carvacrol were treated with 10 cc. of 10% sodium hydroxide solution and to the mixture 20 cc. of boiling water were added. The solution was filtered while hot. A current of carbon dioxide was passed into the alkaline solution precipitating the oxide of dihydroxymercuri-carvacrol. The precipitate was filtered off, washed with alcohol and water and then dried. The precipitate appeared as a fine, yellowish white amorphous powder which decomposed without fusion at 223–250° C.

Results of the analysis:

9.259 mg. subs.: 7.003 mg. CO_2 and 1.690 mg. H_2O.

		C	H
Calcd.:	$C_{10}H_{12}OHg_2.H_2O$	20.57	2.42
Found:		20.64	2.04

SUMMARY

From the results of the experiments, it can be concluded that a disubstituted product is produced when carvacrol is made to react with mercuric acetate. It appears that, following the method of Rupp, nitration may be used to determine the positions occupied by the acetoxymercuri groups in the carvacrol molecule.

The curative effects of dichlormercuri-carvacrol in skin infections have been reported by Burt (4). Galenical preparations of this compound in the form of ointments are being tried here and indications point to some possibilities for the compound to find a place in medicine as a remedy for skin diseases and burns.

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Assay of Mercuric Chloride

*By Berl S. Alstodt**

Much of the discussion which follows is applicable to other mercury compounds which are generally determined as the sulfide. Some of the work on ammoniated mercury already completed in this laboratory and which will be presented at some future time tends to confirm the conclusions of this study.

The study was prompted by an effort to avoid the use of hydrogen sulfide in the official assay of mercuric chloride. The use of organic compounds which evolve hydrogen sulfide vapors was also to be avoided. It was believed that thiourea would be suitable for this purpose, since thiourea hydrolyzes in an alkaline medium according to the following equation given by Taylor (1):

$$CS\begin{matrix}NH_2\\NH_2\end{matrix} + HOH + OH^- \longrightarrow S^= + CNS^- + NH_3$$

This reaction does not produce any perceptible odor of hydrogen sulfide.

The thiourea procedure was developed and the utilization of the method was found to be dependent on controlling the p_H of the solution during precipitation. If the solution was too alkaline, the results were low and failed to show any degree of reproducibility. The low results are due probably to the solution of mercuric sulfide, forming the complex ion, HgS_2^{--}. If the p_H of the solution is controlled so as to render it sufficiently alkaline for hydrolysis according to the above equation, the thiourea method yields precise results, but invariably the results are high. In twenty-five determinations by the thiourea method all results ran over 100%. The precision of the series of determinations was about one per thousand.

Since the results were high by the thiourea method, the mercuric chloride was assayed according to the method of U. S. P. XI. The results, presented in Table I, indicate satisfactory precision, but, as in the thiourea method, exceed 100%. The high results, by both the thiourea method and the U. S. P. method, indicate a constant error in the sulfide precipitation of mercuric chloride. Bassett (2) states that the sulfide determination of mercuric chloride may be high by a few tenths of a per cent.

The U. S. P. directs that the precipitate of mercuric sulfide be treated with carbon tetrachloride for one-half hour, that the carbon tetrachloride be drained and that the precipitate of mercuric sulfide be treated with further portions of carbon tetrachloride, until on evaporating a 1-cc. portion of the filtrate, no visible residue remains. In spite of this treatment, the results are high. The treatment was, therefore, modified so that the precipitate of mercuric sulfide was treated with carbon disulfide from one to twenty-four hours in an arrangement described by Scott (3), whereby the precipitate is subjected to continuous extraction.

Table II shows that the average value was only slightly less when extracted with carbon disulfide than when extracted with carbon tetrachloride, although sulfur is considerably more soluble in the former. This would suggest that the amount of free sulfur present is quite small and that the amount is readily extracted by either carbon tetrachloride or carbon disulfide. If the amount of free sulfur is as small as is indicated, then treatment with either solvent may be unnecessary. A series of determinations was, therefore, made according to the U. S. P., except that the precipitated mercuric sulfide was not treated with carbon tetrachloride, nor was it treated with carbon disulfide. Table II includes the result of this series of nine determinations. The result was about

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one per thousand greater than that obtained by the U. S. P. method, and only about two per thousand greater than the method in which the sulfur is extracted with carbon disulfide. This would indicate that if the high results are to be attributed to the precipitation of sulfur, then most of the sulfur must be in a form which is insoluble in carbon disulfide or carbon tetrachloride, or that the sulfur must be so coprecipitated that it cannot be extracted by merely washing with solvents for sulfur. The latter may be the more probable explanation.

The mercuric chloride was assayed according to the British Pharmacopœia, 1932. The results, Table I, show satisfactory precision. The method is considerably more rapid than the gravimetric procedure and is devoid of any difficulties. It is recommended, however, that the sample of mercuric chloride taken for analysis by the B. P. method should be about 0.5 Gm., rather than 0.3 Gm., since an error of 0.05 cc., which is readily possible in a residual titration, causes a variation of 0.23% for the smaller sample and only about 0.14% for the larger sample.

Since the results were much lower by the method of the British Pharmacopœia than by the method of the United States Pharmacopœia, it was deemed advisable to check the assay by some other method. For this purpose the Rauscher (4) method was adopted. The Rauscher volumetric method, whereby the mercury compound is reduced to metallic mercury, which is subsequently dissolved and titrated with a thiocyanate solution, is modified in that the reduction is carried out at 170–180° C. for 15 minutes by heating in an oil-bath, as recommended by Shukis and Tallman (5). It was found, however, that even the latter method, which improved the original method, leaves tiny particles of mercury which remain on the surface of the monoethanolamine and are lost when the mercury is washed. To overcome this objection, the procedure was modified so that the reaction is carried out in a 500-cc. iodine flask, fitted with a ground 100-cm. condenser tube. The mercuric chloride is reduced in this flask by heating with monoethanolamine in a paraffin bath between 170–180° C. for 15 minutes. When reduction is complete, the supernatant liquid is decanted through a small filter paper and the nitric acid necessary to dissolve the mercury is passed through the same filter, so that any particles of mercury on the filter paper will be recovered.

In the Rauscher method the potassium permanganate, used to ensure the absence of nitrous acid and mercurous mercury, is destroyed by a dilute solution of hydrogen peroxide. This was modified in that the permanganate is destroyed by a dilute solution of ferrous sulfate, which, being oxidized to ferric ion, subsequently may serve as the indicator during the titration with ammonium thiocyanate.

EXPERIMENTAL

Thiourea Method.—Dissolve about 0.5 Gm. of mercuric chloride, accurately weighed, in 300 cc. of distilled water containing 10 cc. of 1*N* hydrochloric acid. Add 5 drops of methyl red test solution and render just alkaline with 3*N* sodium hydroxide. Heat the solution to almost boiling and add from a burette dropwise with constant stirring 50 cc. of 0.02*M* thiourea solution, gently boiling the solution during the addition of the reagent. Set aside for about one to two minutes and observe whether the supernatant liquid is yellow. If the solution is not yellow, or if the precipitate fails to settle during this time, add 3*N* sodium hydroxide until the solution is distinctly yellow, and boil for another minute. This treatment should be repeated until the supernatant liquid remains yellow when the precipitate settles. Let digest for about one hour and filter through a sintered crucible, containing a small stirring rod. Wash the precipitate of mercuric sulfide with hot water until free of chlorides. Then wash with two 10-cc. portions of alcohol. Add 10 cc. of carbon disulfide, stir well, let stand for about five minutes and then suck through. Treat in a similar fashion with another 10-cc. portion of carbon disulfide. Apply strong suction for about one minute. Treat with 10 cc. of alcohol, mix well and suck the alcohol through. Treat with another 5-cc. portion of alcohol. In similar manner treat with two 5-cc. portions of ether. Apply strong suction for about one to two minutes and dry for one-half hour in an oven at 110° C. Repeat drying to constant weight. The weight of mercuric sulfide multiplied by 1.167 represents the equivalent of mercuric chloride in the sample taken for analysis.

U. S. P. XI Method.—Follow the Pharmacopœia as directed under "Hydrargyri Bichloridum," collecting the precipitate in a sintered crucible (1G3) containing a small stirring rod, and from there on proceed as directed under the Thiourea method with, "Wash the precipitate of mercuric sulfide

with hot water . . . ." The calculations are identical with those given under the Thiourea method.

British Pharmacopœia Method.—Follow the method as directed in the British Pharmacopœia, 1932, under "Hydrargyri Perchloridum," using 0.5 Gm. of mercuric chloride, 100 cc. of distilled water, 15 cc. of solution of potassium iodide, 5 cc. of solution of formaldehyde, 20 cc. of solution of sodium hydroxide, 25 cc. of acetic acid and 50 cc. of the volumetric solution of iodine. Otherwise, follow the method as given in the British Pharmacopœia. One cubic centimeter of 0.1*N* iodine solution is equivalent to 0.01358 Gm. of mercuric chloride.

Table I.—Determination of Mercuric Chloride by Various Methods

	Thiourea		U. S. P. XI		B. P. 1932	Rauscher
	CCl_4	CS_2	CCl_4	CS_2		
Per Cent of $HgCl_2$	100.45	100.41	100.31	100.24	99.49	99.54
Av. Dev. of a Single Detn.	0.09	0.11	0.14	0.12	0.14	0.16
No. of Detns.	10	15	26	21	10	12

Rauscher's Method.—Transfer about 0.5 Gm. of mercuric chloride, accurately weighed, to a 500-cc. iodine flask, fitted with a ground 100-cm. condenser tube. Add 5 cc. of purified monoethanolamine. Insert the condenser tube, moistening the ground portion with a trace of monoethanolamine, and place 1 cc. of the reagent in the lip of the iodine flask. Heat in an oil or paraffin bath between 170–180° C. for fifteen minutes. Remove the flask from the bath, let cool and wash down the tube and the flask with 100 cc. of water. Disconnect the condenser tube and filter the liquid through a small filter paper, retaining the globule of mercury in the flask. Wash the flask repeatedly with water until the washings are free of chlorides, passing the washings through the filter. Through the filter pass 10 cc. of 16*M* nitric acid and collect it in the iodine flask containing the globule of mercury. Let stand until the mercury is completely dissolved. Wash the filter with several small portions of water, adding the washings to the mercuric nitrate solution. Add 5% potassium permanganate until the solution remains pink for one to two minutes. Add a 1% solution of ferrous sulfate until the permanganate is just decolorized. Dilute with 200 cc. of distilled water, add 2 cc. of ferric ammonium sulfate test solution, and titrate with 0.1*N* ammonium thiocyanate. One cubic centimeter of 0.1*N* ammonium thiocyanate solution is equivalent to 0.01358 Gm. of mercuric chloride.

Table II.—Hydrogen Sulfide Determinations of Mercuric Chloride

	No. of Detn.	Per Cent of $HgCl_2$	Av. Dev. of Sing. Detn.
Washed with CCl_4	26	100.31	0.14
Washed with CS_2	21	100.24	0.12
No washing with CCl_4 or CS_2	9	100.44	0.24

SUMMARY

1. To avoid the vapors of hydrogen sulfide, the hydrolysis of thiourea is used to precipitate mercuric chloride as mercuric sulfide. The method shows satisfactory precision, but yields high results.

2. The U. S. P. method for determining mercuric chloride likewise gives high results. Washing the sulfide precipitate with carbon disulfide instead of carbon tetrachloride does not remedy the difficulty. If the precipitate is not washed with carbon disulfide or with carbon tetrachloride the results are only slightly worse.

3. The B. P. method is satisfactory and obviates the difficulties entailed in the sulfide procedures. It is the most rapid of the methods studied.

4. The Rauscher volumetric method is modified so as to overcome any losses of mercury. The method is satisfactory and preferable to the B. P. method in that it is a direct rather than a residual titration.

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"You can know little of any idea until you know the history of that idea."—AUGUSTA COMTE.

The Determination of Some Physical and Chemical Constants of Certain Detergents

By B. L. Holiday, E. A. Kelly and L. W. Rising

While much work has been done in an effort to clarify the chemistry of detergents to the end that a better understanding may be reached of how these substances function as cleansing agents, the precise answers are still lacking.

Certain advantages are claimed for individual detergents based on alleged differences in makeup. These claims have commercial significance and should therefore be supportable by factual evidence. The chemistry of soaps is sufficiently well advanced to indicate probable difficulty in justifying divergent advertising claims, particularly for products in the same price class.

Because of the facts stated in the preceding paragraphs, the study to be described in these and succeeding papers was initiated. It is hoped that some contribution can be made which will aid in building up a more comprehensive knowledge of the chemical and physical factors in the act of removing soil and to develop a clear-cut and practical comparison between the detergents now available commercially.

EXPERIMENTAL

This investigation was begun with the purchase of ten varieties of toilet soaps sold widely in drug stores. Samples of these were subjected to a series of experiments designed to compare them on the basis of their physical and chemical properties.

First, their content of volatile material was determined. The total volatile matter contained was ascertained by means of the A. O. A. C. heat method (1). The common xylene method of the A. O. A. C. (2) gave the amount of moisture present. The non-moisture volatile matter was calculated in the usual way by difference. Table I shows the results of these determinations. In it the soaps are listed beginning with the one having the highest content of total volatile matter.

Table I.—Volatile Material Contained by the Soaps Tested

Soap	Per Cent of Total Volatile Matter	Per Cent of Moisture	Per Cent of Volatile Material Not Moisture
G	8.10	7.87	0.23
D	7.14	6.50	0.64
B	6.38	6.24	0.14
H	6.16	5.79	0.37
A	5.98	5.73	0.25
I	4.97	4.54	0.43
F	4.61	4.31	0.30
J	3.90	3.74	0.16
C	3.14	3.10	0.04
E	2.96	2.88	0.08

Plotting the total volatile matter contained by each soap against its percentage of moisture developed the curve shown in Fig. 1. From the graph it would appear that some correlation existed between the two properties used in its formation.

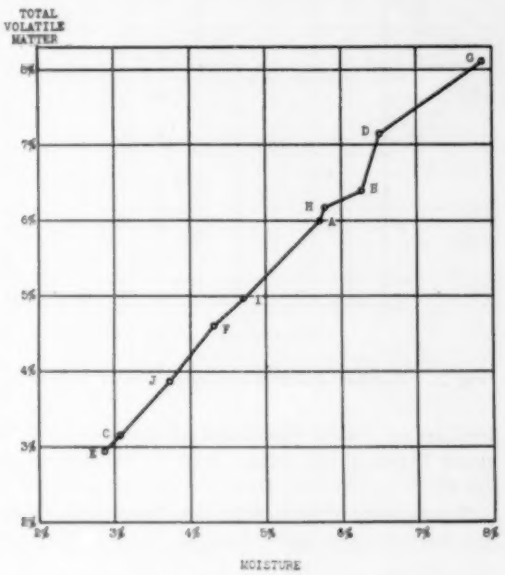

Fig. 1.—Total Volatile Matter and Moisture.

Samples of the soaps were next extracted with alcohol, then with water in order to determine their content of alcohol-soluble and water-soluble material. The residue from the extractions was called the insoluble matter. The data pertaining to these values are expressed in Table II. The soap containing the greatest amount of alcohol-soluble matter is listed first.

Table II.—Alcohol-Soluble, Water-Soluble and Insoluble Matter in the Soaps Studied

Soap	Per Cent of Alcohol-Soluble Matter	Per Cent of Water-Soluble Matter	Per Cent of Insoluble Matter
I	98.13	1.79	0.08
D	95.44	3.04	1.52
G	93.94	5.60	0.46
A	93.76	4.36	1.88
J	92.86	7.07	0.07
C	92.79	4.82	2.39
B	92.69	6.03	1.28
H	89.65	7.12	3.23
E	89.33	8.13	2.54
F	63.94	5.18	30.88

Figure 2 is the result of plotting the percentage of alcohol-soluble matter for each soap against its concentration of water-soluble material. It is

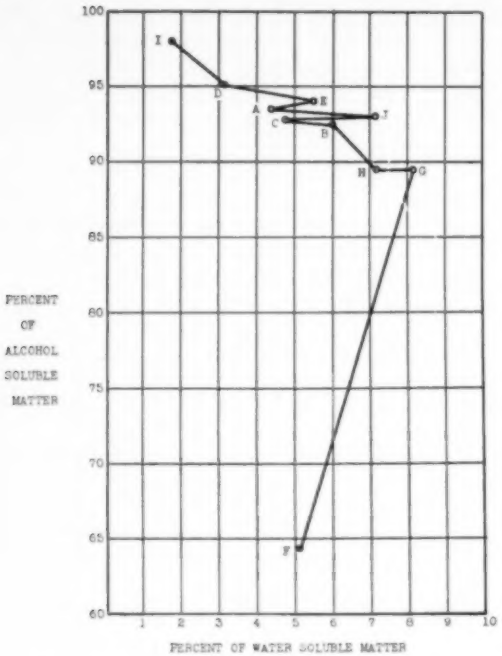

Fig. 2.—Alcohol-Soluble and Water-Soluble Matter.

obvious no feasible mathematical correlation can be made between the values used in developing the curve.

Significance is frequently attached to the hydrogen-ion concentration of soaps in standard dilutions.

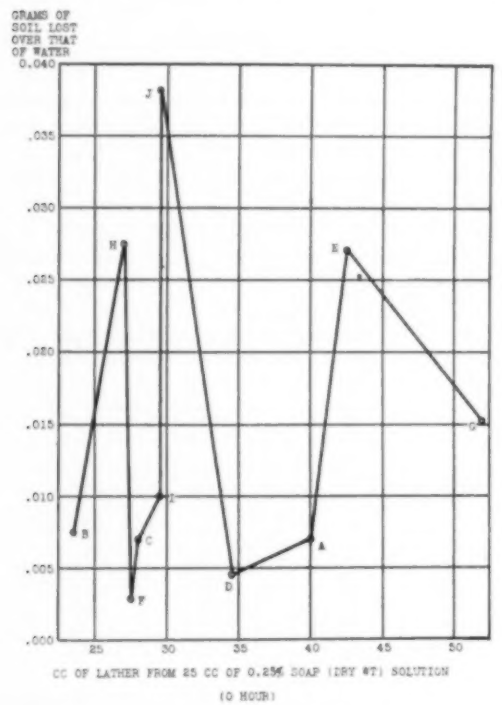

Fig. 3.—Comparison of Soil-Removing Ability with the Property of Seeds Formation.

Therefore, the p_H value of 0.25 per cent solutions of each of the ten soap samples was determined with a Leeds and Northrup potentiometer using a glass electrode. The values obtained are given in Table III. The highest value is listed first.

Table III.—p_H Values of 0.25% Aqueous Solutions of the Soaps

Soap	p_H (25° C.)	Soap	p_H (25° C.)
H	10.5	*E*	10.3
I	10.5	*A*	10.2
J	10.4	*G*	10.1
D	10.4	*B*	9.4
C	10.3	*F*	8.8

Inasmuch as surface tension is an important factor in the efficiency of detergents, its value for the soap samples used in the p_H work was determined. The data were obtained by means of a du Noüy Precision Tensiometer. The results are shown in Table IV where the largest surface tension expressed in dynes is given first.

Table IV.—Surface Tensions of 0.25 Per Cent Aqueous Solutions of the Soaps

Soap	Surface Tension (25° C.) in Dynes	Soap	Surface Tension (25° C.) in Dynes
A	30.5	*I*	28.5
J	29.5	*C*	28.4
G	29.2	*E*	28.1
D	29.1	*F*	27.4
H	29.0	*B*	26.4

The ability to form copious and lasting suds is often taken as a criterion of an efficient soap. To test this property 25 cc. of a 0.25 per cent aqueous solution of each soap at 20° C. were placed in a 100-cc. glass-stoppered graduated cylinder and inverted completely ten times. Readings of the volume in cc. of the suds were recorded immediately after the tenth inversion and at the end of one-, two- and twenty-four-hour periods. Table V shows the results. In it the soap producing immediately the greatest amount of suds is listed first.

Table V.—Height of Suds above the Supporting Columns of 25 Cc. of 0.25% (Dry Weight) Soap Solutions at 20° C.

Soap	0 Hour, Cc.	1 Hour, Cc.	2 Hours, Cc.	24 Hours, Cc.
G	52	42	29.5	4.5
E	42.5	33.5	24.5	0
A	40	31.5	29	5
D	34.5	30	13.8	0
J	29.5	26.5	24.5	4.75
I	29.5	20.5	5	4.5
C	28	25	23	0
F	27.5	23.5	20.5	3.5
B	23.5	21	20	7

An attempt was made to rate efficiencies of the soaps as soil removers. A practical determination of this ability would furnish an effective basis for beginning a correlation, if any is possible, between the efficiency and the previously determined physi-

cal and chemical properties. To this end the various samples were submitted to soil tests in a specially constructed machine.

The apparatus consisted of a 500-cc. glass jar provided with a screw cap. Through the cap and into the jar was fitted a twisted wire agitator which went to and fro as the handle was raised and lowered. The agitator carried a removable tin container for the soil, the size of which was 4 x 4 x 1 cm. The soil used was a 5 per cent ointment of lampblack in tallow.

The test was performed as follows: 240 cc. of 0.25 per cent soap solution were placed in the jar. The previously weighed tin of soil was attached to the agitator, placed in the solution and set in motion at the rate of 240 revolutions per minute at 20° C. The agitation was continued for exactly five minutes. The tin was then removed, partially dried in a current of air and dried in a sulfuric acid desiccator to constant weight. A blank determination using pure water was run. The difference between the loss of weight in the soap solution and that in pure water represented the weight of soil removed by the soap. The resulting data are shown in Table VI.

Table VI.—Detergent Test; Soil Washed for Five Minutes, 240 R. P. M., 20° C. in 240 Cc. of 0.25% Soap Solution

	Average Loss, Gm.	Loss over That of Water, Gm.
Water	0.0508	.....
Soap *J*	0.0891	0.0383
Soap *H*	0.0784	0.0276
Soap *E*	0.0781	0.0273
Soap *G*	0.0660	0.0152
Soap *I*	0.0608	0.0100
Soap *B*	0.0585	0.0077
Soap *A*	0.0579	0.0071
Soap *C*	0.0579	0.0071
Soap *D*	0.0555	0.0047
Soap *F*	0.0538	0.0030

Ability to form copious suds seems to have little relationship with ability to remove soil. Figure 3 where the two properties are plotted against each other shows plainly this lack of relationship.

Alkalinity of the soaps was determined by titrating solutions of one Gm. of each with normal sulfuric acid. The results are shown in Table VII.

For the sake of comparison the total alkalinity and p_H values of the soaps were plotted against each other. Figure 4 is the result.

Table VII.—Number of Cc. of Normal Sulfuric Acid Required to Neutralize the Alkalinity of 1 Gm. of Soap

Soap	No. of Cc. of Normal Acid Required	Soap	No. of Cc. of Normal Acid Required
A	1.3671	*C*	1.0473
I	1.3671	*E*	0.6236
D	1.3111	*G*	0.6076
J	1.2791	*B*	0.5356
H	1.1752	*F*	0.1679

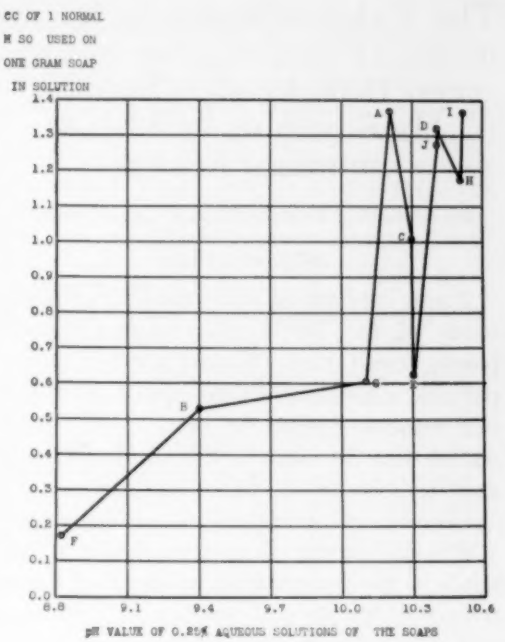

Fig. 4.—Total Alkalinity and p_H Values.

SUMMARY

Ten soaps were examined and the following properties determined for each: percentage of volatile matter, moisture, non-volatile matter, alcohol-soluble matter, water-soluble matter, and insoluble matter, the p_H value, surface tension, ability to form suds, ability to remove soil and total alkalinity.

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Robert Bárány (1876–1936) was awarded the Nobel Prize for physiology and medicine in 1914 for his work on the physiology and pathology of the human vestibular apparatus. To this day, his graduation thesis is regarded as the source of all scientific knowledge on the vestibular apparatus.

The Value of Palisade Ratios in the Differentiation of Official Belladonna, Digitalis, Hyoscyamus and Stramonium Leaves

By Bernard S. Feinstein and Frank J. Slama†*

INTRODUCTION

Zörnig and Weiss (1) in their anatomical study of the leaves of certain composite plants found that the average number of palisade cells beneath an upper epidermal cell was of diagnostic value. They also stated that the ratio of the number of palisade cells included within the area of any epidermal cell remained almost constant. Wallis and Dewar (2) later made a thorough study of the comparative anatomy of leaves belonging to several species of *Barosma*, and they also concluded "that variation in the palisade ratio is independent of the position of the leaf." Furthermore, they were the first to use the term "palisade ratio," stating in their paper that "the total number of palisade cells beneath four upper epidermal cells. . . . when divided by four, gave the recorded figure for the 'palisade ratio,' this being the term which we have adopted to express the average number of palisade cells beneath an upper epidermal cell." Wallis and Dewar also found that the palisade ratio was of value in the differentiation of certain other species of *Barosma* from the official *Barosma betulina* and that it was a useful diagnostic character. Markwell and Cross (3) utilized this diagnostic character as a means of detecting an adulterant of rubbed spearmint leaves.

Later, Dewar (4, 5, 6), working with various species of digitalis, showed that "there is no constant increase or decrease of the ratio in going from the base to the apex of the leaf." Wallis and Forsdike (7) confirmed this constancy of the palisade ratio within a species by their investigation on the leaves of *Atropa belladonna*, *Scopolia carniolica* and *Solanum nigrum;* and, in addition, they concluded that the palisade ratio does not change with the age of the leaf, the habitat or from year to year within any definite species.

Since the palisade ratio appears to be a fairly constant character and of diagnostic value, the authors thought that it may prove to be of important pharmacognostical value in the microscopical differentiation of the leaves of *Atropa belladonna* Linné, *Digitalis purpurea* Linné, *Hyoscyamus niger* Linné and *Datura stramonium* Linné by students.

EXPERIMENTAL

The experimental work consisted of three parts, (*a*) a study of 2–3 mm. squares cut from the apex: center and base of leaves obtained in 1939; (*b*) an examination of powdered (No. 60) four-year-old leaves obtained from stock; and (*c*) an attempt to identify each leaf, or leaves, in unknowns consisting

Table I.—Palisade Ratios of the Leaf Segments of the Four Species

Species	Segment of Leaf	No. of Leaves Examined	No. of Determinations	Average Palisade Ratio	Range of Palisade Ratio
Hyoscyamus niger	Center	2	14	4.6	3.25–5.0
	Base	2	14	5.4	4.25–7.0
	Apex	4	18	5.1	4.25–7.0
			46	(Av.) 5.0	3.25–7.0
Atropa belladonna	Center	3	18	5.9	4.0 –8.7
	Base	2	15	6.9	5.0 –8.5
	Apex	2	15	6.5	4.5 –8.2
			48	(Av.) 6.4	4.0 –8.7
Datura stramonium	Center	2	17	5.3	3.75–7.5
	Base	4	21	5.1	2.75–6.7
	Apex	2	16	5.0	3.5 –8.5
			54	(Av.) 5.1	2.75–8.5
Digitalis purpurea	Center	3	22	4.3	3.5 –5.2
	Base	2	19	3.9	2.0 –6.2
	Apex	2	17	4.0	3.0 –5.0
			58	(Av.) 4.1	2.0 –6.2

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of either an unadulterated powder or of mixtures of two or more species.

The clearing solution which was used throughout this work consisted of 50 Gm. of chloral hydrate dissolved in 20 cc. of distilled water. The 2–3 mm. squares and the powders were cleared by boiling in a test-tube on a water-bath for 15 minutes, the material being completely covered by the clearing solution. The material was then mounted in the clearing solution and examined under a magnification of 450×. In each determination the number of palisade cells under any four adjacent epidermal cells, when divided by four, represented the palisade ratio; and in no case was any palisade cell included where more than one-half of its area lay outside of the epidermal cells.

The results obtained from the study of the leaf segments of the four species are shown in Table I.

Table II.—Palisade Ratios of the Four Species of Powdered Leaves

Species	No. of Determinations	Average Palisade Ratio	Range of Palisade Ratio
Hyoscyamus niger	15	4.7	4.0 –5.2
Atropa belladonna	17	6.5	5.7 –9.5
Datura stramonium	18	5.0	4.0 –7.0
Digitalis purpurea	14	3.8	2.75–4.7

Table III.—Palisade Ratios of the Unknown Powdered Species

Unknown	Specimen Examined	No. of Determinations per Specimen	Average Palisade Ratio	Range of Palisade Ratio
No. 1 (digitalis)	*a*	6	3.75	3.25–40
	b	7	4.75	4.25–5.2
	c	6	4.75	4.0 –5.25
	d	5	5.75	5.5 –6.0
	e	7	4.30	4.0 –5.0
	f	7	3.80	3.5 –4.25
	g	8	4.75	4.0 –5.75
No. 2 (digitalis and hyoscyamus)	*a*	7	3.75	3.0 –4.5
	b	5	4.30	4.0 –4.75
	c	4	3.74	3.25–4.0
	d	5	4.25	3.75–4.5
	e	5	3.50	2.5 –3.75
	f	5	4.25	3.75–5.0
	g	5	4.3	4.0 –4.75
No. 3 (digitalis, hyoscyamus, stramonium and belladonna)	*a*	5	7.0	5.75–8.25
	b	5	4.5	4.0 –5.0
	c	5	3.6	3.25–4.5
	d	6	5.75	4.5 –7.0
	e	5	3.6	2.5 –4.25
	f	5	7.25	6.25–8.25
	g	5	6.5	5.25–7.75
	h	5	4.25	3.5 –5.5
	i	5	4.2	3.5 –5.0
	j	5	4.8	4.0 –5.25
	k	5	5.4	4.25–6.0

From a study of Table I, it is readily seen that the average palisade ratio of any leaf does not differ sufficiently from those of the other species to be of value in differentiating all of the four species studied. However, it appears to be possible to distinguish belladonna leaves from the remaining three species because of their somewhat higher palisade ratio. This has been successfully carried out by the authors.

The results of the study of the powdered leaves in Table II show that the average palisade ratios of the powdered belladonna, digitalis, hyoscyamus and stramonium leaves are of little practical value in the differentiation of the four powders—with the possible exception of belladonna leaves.

In order to confirm the results obtained in Table II, unknowns shown in Table III were studied.

From the results in Table III, it is shown that the palisade ratios cannot be utilized to distinguish between digitalis, hyoscyamus and stramonium leaves. However, as seen in Unknown No. 3, it is possible to differentiate the belladonna leaf as it has a higher average palisade ratio (6.5–7.25) when compared with the remaining species.

CONCLUSIONS

1. A study has been made of the palisade ratios of the official leaves of belladonna, digitalis, hyoscyamus and stramonium.

2. The palisade ratios are of practically no value in the differentiation of the official hyoscyamus, digitalis and stramonium leaves.

3. It is possible to use the palisade ratio of the official belladonna leaves as a means of differentiating them from hyoscyamus, digitalis and stramonium leaves.

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(2) Wallis and Dewar, *Quart. J. Pharm. Pharmacol.*, 6 (1933), 347.

(3) Markwell and Cross, *Analyst*, 60 (1935), 784.

(4) Dewar, *Quart. J. Pharm. Pharmacol.*, 6 (1933), 448.

(5) Dewar, *Ibid.*, 7 (1934), 15.

(6) Dewar, *Ibid.*, 7 (1934), 342.

(7) Wallis and Forsdike, *Ibid.*, 11 (1938), 700–708.

The Netherlands has honored the following scientists by portraying them on postage stamps: Antonj van Leeuwenhoek, the first microbe hunter; Hermann Boerhaave, founder of the modern clinic; Frans Cornelis Donders, physiologist and ophthalmologist.

A Study of Silica Gel as a Carrier for Antiseptics*,†

*By William A. Prout‡, Mae S. Eddleman** and Rhett G. Harris††*

In 1937, Peronnet and Genet (1) reported that silica gel as an ointment base had certain characteristics which indicated to us its potential possibilities as a carrier for certain germicidal substances. It was shown by these investigators that silica gel formed with glycerin a stable ointment base which could be kept over a period of several months without deterioration and which might be used in the manufacture of certain ointments which are official in the pharmacopœia of France. Upon the assumption that a satisfactory ointment base had been perfected, we have attempted to evaluate the efficiency of the silica gel and glycerin and other silica gel bases as carriers of certain antiseptic medicaments by comparing the bacteriocidal action of the ointments made with these silica gel bases with the bacteriocidal action of the same antiseptic medicaments when incorporated in the base now official in the United States Pharmacopœia XI.

Ointments made with Base "A"
(Silica Gel and Glycerin)

ZnO	1.50
H_3BO_3	8.40
C_6H_5OH	10.25
$HgNH_2Cl$	17.25

Zone in mm.

Controls: U. S. P. Base—no zone.
Base "A"—no zone.
U. S. P. ointments ZnO, H_3BO_3 and C_6H_5OH—no zone.
$HgNH_2Cl$—6 mm. zone.

Ointments made with Base "B"
(Silica Gel and Expressed Oil of Almond)

ZnO	1.50
H_3BO_3	8.75
C_6H_5OH	10.60
$HgNH_2Cl$	17.95

Zone in mm.

Controls: U. S. P. Base—no zone.
Base "B"—no zone.
U. S. P. ointments ZnO, H_3BO_3 and C_6H_5OH—no zone.
$HgNH_2Cl$—6 mm. zone.

Ointments made with Base "C"
(Silica Gel and Cottonseed Oil)

ZnO	1.50
H_3BO_3	8.85
C_6H_5OH	9.45
$HgNH_2Cl$	14.95

Controls: U. S. P. Base—no zone.
Base "C"—no zone.
U. S. P. Ointments ZnO, H_3BO_3 and C_6H_5OH—no zone.
$HgNH_2Cl$—6 mm. zone.

Ointments made with Base "D"
(Silica Gel and Olive Oil)

ZnO	1.50
H_3BO_3	8.00
C_6H_5OH	10.55
$HgNH_2Cl$	15.40

Controls: U. S. P. Base—no zone.
Base "D"—no zone.
U. S. P. Ointments ZnO, H_3BO_3 and C_6H_5OH—no zone.
$HgNH_2Cl$—6 mm. zone.

Ointments made with Base "E"
(Silica Gel and Liquid Petrolatum)

ZnO	1.50
H_3BO_3	9.85
C_6H_5OH	8.30
$HgNH_2Cl$	16.65

Controls: U. S. P. Base—no zone.
Base "E"—no zone.
U. S. P. Ointments ZnO, H_3BO_3 and C_6H_5OH—No zone.
$HgNH_2Cl$—6 mm. zone.

Ointments made with Base "F"
(Silica Gel and Castor Oil)

ZnO	1.50
H_3BO_3	8.95
C_6H_5OH	9.95
$HgNH_2Cl$	17.15

Controls: U. S. P. Base—no zone.
Base "F"—no. zone.
U. S. P. Ointments ZnO, H_3BO_3 and C_6H_5OH—No zone.
$HgNH_2Cl$—6 mm. zone.

EXPERIMENTAL

Six different bases were prepared by incorporating silica gel with glycerin, expressed oil of almond, cottonseed oil, olive oil, liquid petrolatum and castor oil, respectively. With each of these six bases, ointments of zinc oxide, ammoniated mercury, phenol and boric acid were prepared. The twenty-four ointments were then compared with the corresponding pharmacopœial ointments, by using the agar cup-plate method for testing antiseptics and

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disinfectants (2), which is one of the accepted methods of the United States Food and Drug Administration. Briefly, the procedure is as follows: 20 cc. of nutrient agar which had been melted and cooled to 42–45° C. and to which had been added 0.1 cc. of a 24-hour broth culture of *Staphylococcus aureus*,[1] were poured into a sterile petri dish and allowed to harden at room temperature. By means of a sterile cork borer, a disk, 1.5 cm. in diameter, was cut out of the solidified agar, leaving a cup with an approximate depth of 0.2 cm. Into this cup was placed 0.5 Gm. of the ointment to be tested, and the plate was then incubated under an unglazed porcelain top for 24 hours. At the end of the period of incubation, the zones of inhibition were measured by means of a vernier caliper. Each ointment was prepared secundum artem, maintaining the pharmacopœial strengths of the active constituents and employing the bases previously mentioned.

The accompanying graphs show the differences between the zones of inhibition produced by the germicidal agents when incorporated in the silica gel bases and the same germicidal agents when incorporated in the pharmacopœial base. It will be be noted that the zones produced by any one of the antiseptic medicaments are approximately the same with all six silica gel bases; that there are significant differences between the individual antiseptic medicaments, which would be expected inasmuch as the germicidal power of the medicaments *per se* varies greatly; that the corresponding ointments prepared with the pharmacopœial base failed, with the exception of ammoniated mercury, to exhibit any zones of inhibition.

DISCUSSION

Earlier investigation of two of the authors (3) has shown that these same antiseptic medicaments when prepared with other bases failed (again with the exception of ammoniated mercury) to exhibit any zones of inhibition when tested in a similar manner. At present the explanation of these observed differences is theoretical and is based upon the apparent constitution of the bases employed.

An ointment that has any antiseptic value owes that characteristic to the fact that the medicament, in sufficient concentration to destroy microörganisms, is capable of diffusing from the base to the surface upon which it is applied. Any factor or factors which have a tendency to influence the diffusion of the medicament from the ointment base will, in turn, affect the antiseptic property of the ointment. It would appear as though silica gel, due to its high water content, permits a greater diffusion of the medicament from the ointment. On the other hand, the pharmacopœial base having a relatively low water content and holding securely what little water is present, apparently does not afford a very good carrier for certain antiseptic substances.

[1] No. 209, obtained from the United States Department of Agriculture.

CONCLUSIONS

1. Silica gel, when combined with such substances as glycerin, expressed oil of almond, liquid petrolatum, castor oil, olive oil and cottonseed oil, respectively, appears to react satisfactorily as a carrier for certain antiseptic medicaments.

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(1) Peronnet and Genet, *J. pharm. chim.*, 26 (1937), 490.

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Phenolic Ointments*

By Carl B. Burnside† and Rudolph A. Kuever‡

INTRODUCTION

Phenol Ointment has long been used as an antiseptic application. It has been official in the United States Pharmacopœia (1) since 1873 at which time it first appeared in the fifth revision under the title, Ointment of Carbolic Acid. This ointment contained 12.5% of phenol. In six revisions of the United States Pharmacopœia the formula for the official ointment was altered as many times. Its phenol content has been gradually reduced until at present it contains 1.8 to 2.2%. The title was changed from Ointment of Carbolic Acid to Ointment of Phenol in the eighth revision and in the eleventh revision it takes the name Phenol Ointment.

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† Abstract from a thesis presented to the Graduate College of the State University of Iowa in partial fulfilment of the requirements for the degree Master of Science.

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The base has been changed at various times and these changes may be noted in the accompanying Table I.

Table I.—Changes in Composition of Phenol Ointment, 1873–1936

U. S. P.	Year	Title	Phenol Content, %	Base, %	
V	1873	Ointment of Carbolic Acid	12.5	Yellow wax Lard	17.5 70.0
VI	1882	Ointment of Carbolic Acid	10.0	Yellow wax Lard	18.0 72.0
VII	1893	Ointment of Carbolic Acid	5.0	Yellow wax Lard	19.0 76.0
VIII	1905	Ointment of Phenol	3.0	White Petrolatum	97.0
IX	1916	Ointment of Phenol	2.25	White wax Benzoinated lard	19.55 78.20
X	1925	Ointment of Phenol	2.0	Yellow wax Petrolatum	5.0 93.0
XI	1936	Phenol Ointment	1.8–2.2	Yellow wax Petrolatum	5.0 93.0

HISTORICAL

In view of the work that has been done with phenol and phenolic derivatives, as it applies to their use in oily base ointments, it is obvious that their antiseptic potency is quite unpredictable. When tested for bactericidal potency the results obtained are usually unsatisfactory. In 1881 Koch (2) advanced the theory that when phenol is dissolved in oil or alcohol it shows no antiseptic properties. This statement has subsequently been upheld by many workers. Prout and Strickland (3), experimenting with various oleaginous bases, found no bactericidal potency when phenol was incorporated into oily bases. Reddish and Wales (4) tested U. S. P. Ointment of Phenol along with some 40 other ointments and found it to possess no antiseptic potency. By testing various phenol ointments Bryan (5) found that at least 10% of phenol was required to produce bactericidal potency. Gottstein (6), in 1889, made the statement that oil-soluble antiseptics, when incorporated into an oily ointment base, possess no antiseptic potency. Cheyne (7), during World War I, suggested an ointment of 20% phenol for use in the British Navy as an antiseptic application. Husa and Radin (8) experimented with Ointment of Phenol and suggested a formula which was later found to be only feebly bacteriostatic by Gershenfeld and Miller (9) who also concluded that 2% phenol in petrolatum or a mixture of petrolatum and anhydrous wool fat displayed no bactericidal action.

A later report by Gershenfeld and Miller (10) suggested the use of water-miscible vanishing cream bases for bactericidal substances. Clark (11) found phenol to be nonantiseptic in oily bases but suggested the use of lauryl sodium sulfate as an emulsifying agent in the preparation of Phenol Ointments. He observed evidence of increased bactericidal action with a base of petrolatum containing 2.5% glycerin, 2.5% water and 2% of lauryl sodium sulfate, when he prepared ointments of thymol and chlorthymol. Gershenfeld and Brillhart (12) suggested the inclusion of emulsion bases in ointments of the United States Pharmacopœia and National Formulary because greater bactericidal action could thus be obtained.

Mention should be made of work done by Prout and Smith (13) who observed that when Phenol Ointment is made according to the United States Pharmacopœia as much as 25% of its phenol content may be lost by evaporation.

A great deal of work has been done on ointment bases containing emulsifying agents. Formulas are given. Numerous writers have suggested the use of cholesterol and its derivatives (12, 14, 16, 18, 20). Other investigators mention the use of various alcohols for emulsifying purposes (15, 17, 18, 19). Use of hydrogenated castor and peanut oils is suggested by Lesser (16) and Fiero (24). Triethanolamine is suggested by Mumford (15), an English writer.

PURPOSE

Phenol Ointment U. S. P. has no bactericidal potency when tested by the Standard F. D. A. Method (23) using *Staphylococcus aureus* as the test organism. Since phenol is antiseptic it is obvious that an improper base is employed. The purpose of this work is to prepare a Phenol Ointment with bactericidal potency. Since bases like that of U. S. P. Phenol Ointment have proved to be ineffective for phenol, emulsion types of ointment bases were created. Ointments were made with phenols and phenolic de-

rivatives, including phenol, thymol, chlorthymol, resorcinol, hexylresorcinol, chlorcarvacrol, trinitrophenol, *o*-hydroxy diphenyl, *m*-hydroxy diphenyl, *p*-hydroxy diphenyl, 3-chlor-4-hydroxy diphenyl and betanaphthol.

A study was first made of the antiseptic properties of these phenols in U. S. P. Phenol Ointment Base. The same phenols, in the proposed emulsion ointment base, were prepared and their bactericidal potency determined. Considering the fact that U. S. P. Phenol Ointment has a single phase oleaginous base, this affords an opportunity of comparing the action of these same phenols in the two types of bases.

EXPERIMENTAL

Melting points of the various bases employed were determined by using the method devised by Li and Kuever (14).

A tabulation of the melting points of all the U. S. P. Phenol Ointments and of the proposed emulsion ointment base is given in Table II.

Table II.—Melting Points of Phenol Ointments

U. S. P.	Melting Point, Deg. C.
V	49
VI	50
VII	53
VIII	49
IX	53.5
X	47
XI	47
Emulsion ointment base	47

Since Phenol Ointment U. S. P. displays no bactericidal potency, a study was made to determine how the other phenols would behave in U. S. P. Phenol Ointment Base.

Phenol Ointment U. S. P.

Phenol	2 Gm.
Yellow wax	5 Gm.
Petrolatum	93 Gm.

Melt the yellow wax and the phenol on a water-bath, add the petrolatum and stir the mixture until it congeals.

Since Prout and Smith (13) observed a marked loss of phenol by this method, the directions were altered slightly in making these phenolic ointments using the U. S. P. Phenol Ointment Base. In each case two per cent of the phenolic substance used was incorporated into the base using the following directions:

Melt the yellow wax and the petrolatum on a water-bath and stir until nearly congealed before adding the phenolic substance. This method eliminates undue loss of phenols by evaporation.

Table III gives the bacteriological results obtained by testing these ointments using the Standard F. D. A. Method, *Staphylococcus aureus* as the test organism.

Table III.—Bactericidal Potency of Phenolic Ointments (U. S. P.) Made with Phenol Ointment Base

Ointment	Per Cent	Base, %	Zone, Mm.
Hexylresorcinol	2	Yellow wax 5 Petrolatum 93	9.0
Chlorcarvacrol	2	Yellow wax 5 Petrolatum 93	5.0
m-Hydroxy diphenyl	2	Yellow wax 5 Petrolatum 93	5.0
Chlorothymol	2	Yellow wax 5 Petrolatum 93	4.5
Thymol	2	Yellow wax 5 Petrolatum 93	3.0
3-Chlor-4-hydroxy diphenyl	2	Yellow wax 5 Petrolatum 93	3.0
o-Hydroxy diphenyl	2	Yellow wax 5 Petrolatum 93	2.5
Betanaphthol	2	Yellow wax 5 Petrolatum 93	1.5
p-Hydroxy diphenyl	2	Yellow wax 5 Petrolatum 93	No zone
Resorcinol	2	Yellow wax 5 Petrolatum 93	No zone
Trinitrophenol	2	Yellow wax 5 Petrolatum 93	No zone
Phenol	2	Yellow wax 5 Petrolatum 93	No zone

Procedure for Testing Ointments.—The Standard F. D. A. Agar Cup Method (23) was used in each case. The amount of ointment used and the area of contact with the agar in this method are more nearly constant than in the Agar Plate Method. A twenty-four-hour broth culture of *Staphylococcus aureus* in nutrient agar, containing peptone, Leibig's beef extract and salt, as suggested by the F. D. A. Method, was used.

Emulsion Ointment Bases.—Phenol Ointment U. S. P. has a single-phase oleaginous base. This appears to be the reason for its non-antiseptic properties. Oily, single-phase ointment bases, generally are unsatisfactory for phenols. From the results

Fig. 1.—Phenol Ointment U. S. P. XI. Crystals of Phenol May Clearly Be Seen. × 160.

obtained it appears that single-phase oleaginous bases impair the antiseptic power of all the phenols.

Figure 1 is a microphotograph of U. S. P. Phenol Ointment showing that the phenol has crystallized. While undoubtedly the phenol was in solution when the ointment was warm, upon cooling and standing the phenol crystallized as the microphotograph clearly reveals.

Other phenolic ointments displayed similar characteristics with U. S. P. Phenol Ointment base. Ointments employing the proposed emulion ointment base do not show this crystallization. This ointment base has an internal oleaginous and an external aqueous phase. It is evident that the phenols are in solution in the aqueous phase.

Fig. 2.—Product: Phenol Ointment U. S. P. XI.
Test organism: *Staphylococcus aureus* of 24-hour broth culture.
Method: Agar Cup.
Period of Incubation: 48 hours at 37° C.
Width of Zone: No zone.

After some experimentation, the following base was devised as a satisfactory emulsion ointment base.

Gardinol*	0.25 Gm.
Propylene glycol	6.00 Gm.
Water	1.92 Gm.
White petrolatum	91.83 Gm.
To make	100.00 Gm.

* A mixture of neutralized, sulfated, higher alcohols such as lauryl sodium sulfate and oleyl sodium sulfate sold in commerce as Gardinol.

The ointments of the various phenols may be made with the proposed emulsion ointment base according to the following formula:

Phenol	2.00 Gm.
Gardinol	0.24 Gm.
Propylene glycol	5.88 Gm.
Water	1.88 Gm.
White petrolatum	90.00 Gm.
To make	100.00 Gm.

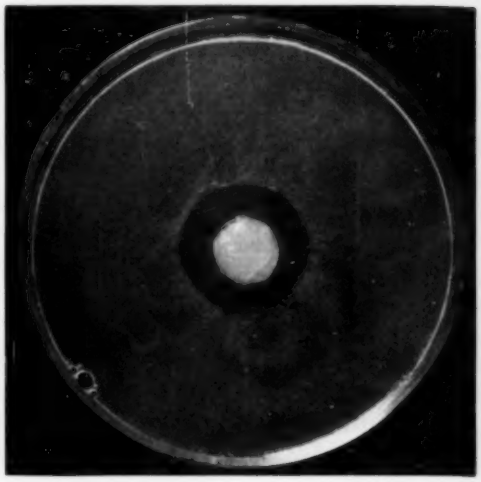

Fig. 3.—Product: Phenol Ointment with Proposed Emulsion Ointment Base.
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.
Method: Agar Cup.
Period of Incubation: 48 hours at 37° C.
Width of Zone: 6.0 mm.

Dissolve the gardinol in the propylene glycol and the water, warming gently if necessary. To this solution add the phenol and agitate or triturate until dissolved or dispersed. Gradually add this phase to the white petrolatum with trituration. A homogeneous emulsion ointment is readily formed.

Resorcinol, *o*-hydroxy diphenyl, phenol, *m*-hydroxy diphenyl, thymol, chlorcarvacrol, chlor-

Fig. 4.—Product: Thymol Ointment with U. S. P. Phenol Ointment Base.
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.
Method: Agar Cup.
Period of Incubation: 48 hours at 37° C.
Width of Zone: 3.0 mm.

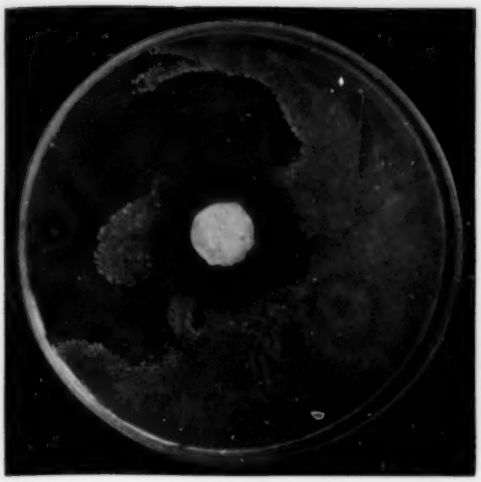

Fig. 5.—Product: Thymol Ointment with Proposed Emulsion Ointment Base.
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.
Method: Agar Cup.
Period of Incubation: 48 hours at 37° C.
Width of Zone: 7.0 mm.

thymol and hexylresorcinol were all completely soluble; 3-chlor-4-hydroxy diphenyl was partially soluble; trinitrophenol, *p*-hydroxy diphenyl and betanaphthol were sparingly soluble in the aqueous phase.

As a matter of comparison twelve ointments were prepared with the proposed emulsion ointment base containing the phenols and phenolic derivatives mentioned in Table III. Their bactericidal potency was determined by the Standard F. D. A. Method. Table IV shows the results.

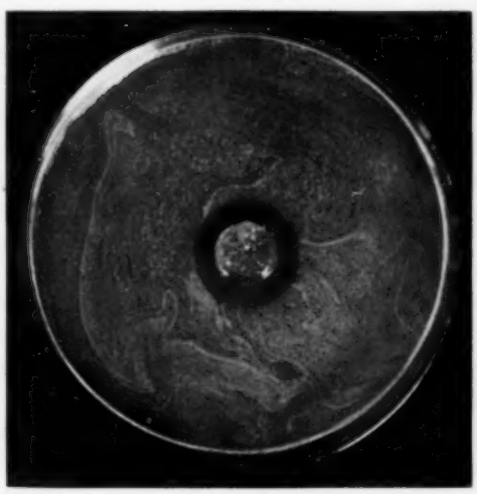

Fig. 6.—Product: Chlorthymol Ointment with U. S. P. Phenol Ointment Base.
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.
Method: Agar Cup.
Period of Incubation: 48 hours at 37° C.
Width of Zone: 4.5 mm.

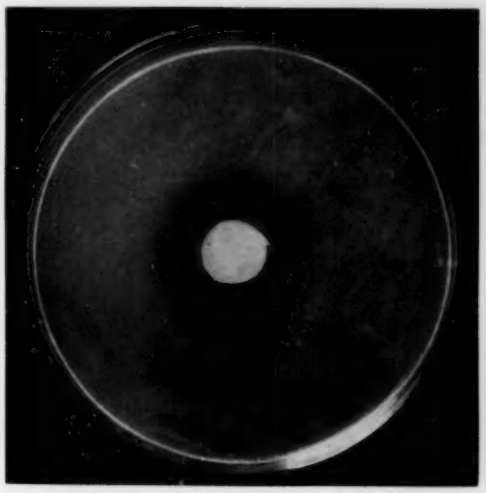

Fig. 7.—Product: Chlorthymol Ointment with Proposed Emulsion Ointment Base.
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.
Method: Agar Cup.
Period of Incubation: 48 hours at 37° C.
Width of Zone: 8.0 mm.

Table IV.—Bactericidal Potency of Phenolic Ointments with Proposed Emulsion Ointment Base

Ointment	Per Cent	p_H	Zone, Mm.
Hexylresorcinol	2	4.8	12.0
Chlorthymol	2	5.1	8.0
Chlorcarvacrol	2	4.6	7.0
Thymol	2	5.0	7.0
m-Hydroxy diphenyl	2	4.9	7.0
Phenol	2	4.6	6.0
Betanaphthol	2	4.8	6.0
3-Chlor-4-hydroxy diphenyl	2	4.8	3.0
o-Hydroxy diphenyl	2	5.2	4.0
Resorcinol	2	4.9	3.0
p-Hydroxy diphenyl	2	5.2	2.5
Trinitrophenol	2	Acid	1.0

p_H *Indices.*—Many antiseptics are activated by acid media. Goedrich (21) and Bittenbender, Degering and Tetrault (22) have shown that antiseptics are more potent when they are in a solution with a p_H index below seven. They have established definite relationships between p_H indices and bactericidal potency.

Three phenol ointments with emulsion ointment bases were prepared, the external phase of which was oleaginous and the internal aqueous. Each ointment contained 2% phenol. The aqueous phase of one was made acid with benzoic acid to a p_H of 3. The aqueous phase of another was left with its natural acidity, a p_H of 4.6; the aqueous phase of the third was made alkaline with sodium borate, to a p_H of 8. Table V shows the relative potency of these three ointments.

Table V.—Bactericidal Potency of Phenol Ointments with Adjusted p_H

Ointment, %	p_H	Zone, Mm.
Phenol 2	3.0	2.0
Phenol 2	4.6	No zone
Phenol 2	8.0	No zone

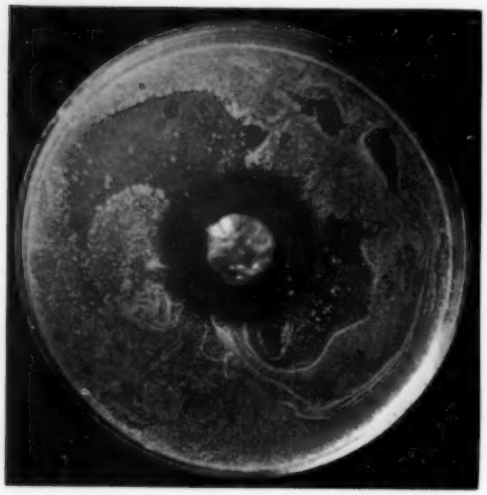

Fig. 8.—Product: Hexylresorcinol Ointment with U. S. P. Phenol Ointment Base.
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.
Method: Agar Cup.
Period of Incubation: 48 hours at 37° C.
Width of Zone: 9.0 mm.

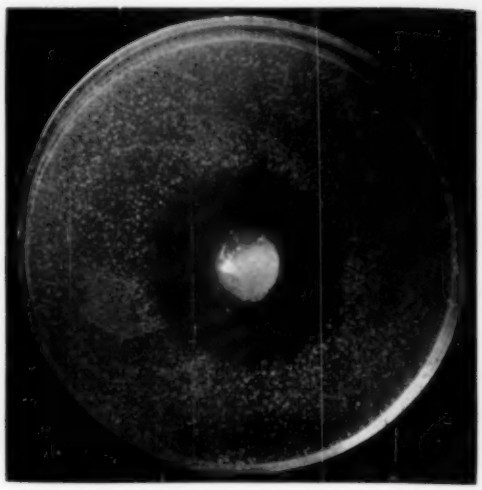

Fig. 9.—Product: Hexylresorcinol Ointment with Proposed Emulsion Ointment Base.
Test Organism: *Staphylococcus aureus* of 24-hour broth culture.
Method: Agar Cup.
Period of Incubation: 48 hours at 37° C.
Width of Zone: 12.0 mm.

The aqueous phases of all the phenols and phenolic derivatives employed in the ointments given in Table IV were acidic in themselves. Their p_H indices are given.

Addition of Waxes to Ointment Bases.—The proposed emulsion ointment base contains no waxes. Its consistency is such that waxes are not required. Incidentally, the addition of waxes to ointments exerts an inhibitory effect on their bactericidal potency. Several waxes including hydrogenated castor oil (Opal Wax), paraffin, white wax, yellow wax, spermaceti and carnauba wax were investigated.

Three ointments of chlorthymol in a base of white petrolatum proved this inhibitory effect clearly. By varying the amounts of white wax and spermaceti in the ointment base, the zones of inhibition varied inversely with the amount of waxes present. Table VI gives the percentages of waxes in these bases and their antiseptic potencies.

Table VI.—Bactericidal Potency of Ointments with Wax Added to the Base

Base, %	Chlorthymol, %	Zone, Mm.
White petrolatum, 90 White wax, 5 Spermaceti, 5	2	1.5
White petrolatum, 95 White wax, 2.5 Spermaceti, 2.5	2	5.0
White petrolatum, 98 White wax, 1 Spermaceti, 1	2	7.5

Previously Husa and Radin (8) and Clark (11) had observed the deleterious effect of waxes. It now becomes apparent how directly and extensively they influence bactericidal properties of ointments.

Figures 2 to 9 show the difference in bactericidal potency of ointments made with U. S. P. Phenol Ointment base and the proposed emulsion ointment base. The width of inhibited zone, measured in millimeters, denotes the antiseptic power of the ointment.

SUMMARY

Phenol Ointment has repeatedly been proved to be non-antiseptic when tested by the Official F. D. A. Method using *Staphylococcus aureus*. This fact has again been confirmed.

Ointments of hexylresorcinol, chlorcarvacrol, *m*-hydroxy diphenyl, chlorthymol, thymol, 3-chlor-4-hydroxy diphenyl, *o*-hydroxy diphenyl and betanaphthol show varying antiseptic potency in the U. S. P. Phenol Ointment base.

Like phenol, *p*-hydroxy diphenyl, resorcinol and trinitrophenol show no antiseptic potency in the U. S. P. Phenol Ointment base.

The twelve phenols and phenolic derivatives all show substantial antiseptic potency in the proposed emulsion ointment base.

Waxes in ointment bases are deleterious by reducing the bactericidal potency of the ointment.

Phenol Ointment U. S. P. XI is devoid of antiseptic potency while the proposed oint-

ment produces a clear zone of inhibition 6 mm. wide.

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Establishment of the Drug Laboratory in the Bureau of Chemistry, United States Department of Agriculture*

By Lyman F. Kebler†

At various times, it has been suggested that the establishment and the early work of the Drug Laboratory of the Bureau of Chemistry of the U. S. Department of Agriculture ought to be written up and that I, being the only living person who is in possession of the necessary information, should write the story. This I have consistently hesitated to do heretofore for the simple reason that it necessarily brings me prominently into the picture. Of late I have, however, decided to put aside my personal feeling in the matter and write up the founding of this laboratory, including some of its early work, some of the prior activities of Congress and the lack of action on the part of Government officials in the field of pure and safe drugs for the suffering sick.

The first Congress of the United States in the second tariff act (1) included "Medicinal Drugs," among the imported articles to pay duty. Drugs used for dyeing were not included under this head. It also provided for the inspection and testing of all kinds of wines. The alcohol content was the chief factor to be considered. Determining the strength of alcohol was included in the first tariff act and all later general tariff acts. During the third session of the same Congress, an extensive tariff law was passed (2) containing 62 sections. Drugs as heretofore were included. A large proportion of the drugs in the early years of our country were imported. Cosmetics and perfumes were covered in the 1794 tariff act. General tariff laws were passed at irregular intervals by more than a score of Congresses. But, excepting the alcoholics, there was apparently a dearth of activity as to the character

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of drugs imported for about half a century. Yet we know from the published records of private investigators that adulterated, low grade and spurious drugs were coming into this country. This in time duly aroused both physicians and druggists. Opium was very commonly adulterated and of low grade, and opium, in those days and for many years later, was the physician's right hand. In time these rogueries were brought to the attention of Congress, and this resulted in the passage of the 1848 drug import law (3), which prohibited the importation of the above character of drugs. The law was administered by the Secretary of the Treasury. Politics seemed to have an early inning. The officials, it was alleged, seemed to be more interested in collecting revenue than in pure and safe drugs for the suffering sick. In spite of this, however, some very good work was done (4) under this law. It stimulated action along various lines, *e. g.*, the creation of standards and methods of analysis, which in turn called for better qualified examiners and better educational facilities.

The appropriation act of 1889 for the Department of Agriculture authorized the Secretary to investigate the adulteration of drugs. Succeeding Congresses granted the same authority to the Secretary over a period of years, but no action was taken, in the case of drugs, under this authority. In 1890, Senator George F. Edmunds' bill, prohibiting the importation of adulterated foods and drugs, became a law (5). Its enforcement was also vested in the Secretary of Agriculture. However, the drug portion was allowed to sleep, largely, I am informed, because Congress did not provide sufficient funds. In 1899 the Secretary of Agriculture was directed (6) to inspect, examine, test and, through the Secretary of the Treasury, refuse entry to any foods or drugs found adulterated and dangerous to health. The same authority was given in several later acts, but still the drug portion was allowed to remain dormant. Through all these years, there was marked agitation for a National Pure Food and Drug Law, and the Department figured prominently therein. So did the pharmacists. The drug interests therefore naturally became skeptical as to anything ever being done in behalf of pure drugs by the Secretary of Agriculture. But things took a turn for the better.

The 1901 appropriation act for the Department of Agriculture provided for raising the Division of Chemistry to the rank of a Bureau. The change was made July 1901. The chemist in his report for the fiscal year ending June 30, 1902, says in part: "The second line of work which the Bureau will undertake will be in the study of drugs. The work will be undertaken in hearty collaboration with the AMERICAN PHARMACEUTICAL ASSOCIATION, which already has a committee studying drug adulteration and the best methods of ascertaining them. The work will be divided into two portions: first, a study of the best methods of assaying drugs, and second, a study of the drug products bought on the open market, to determine their composition and the degree and extent of their adulteration. It is expected that the work will be fully inaugurated during the present year."

The Drug Laboratory was authorized about the same time (7). The first definite information received by the outside world, regarding the establishment of such a laboratory, was a letter of Dr. H. W. Wiley, sent to Professor John Uri Lloyd, on the letter head of the Bureau of Chemistry, June 16, 1902. It reads:

"We are about to undertake, under authority of Congress, a study of the composition and adulteration of drugs. We want to get a man of good training and some experience for the work. Could you suggest the names of some men who you think would render us faithful service and who are available? We can pay $2000 or $2500 per annum for a good, well-trained man. I would be glad to have suggestions from you in this matter.

Thanking you in advance for this courtesy, I am,

Faithfully,

H. W. WILEY, *Chief*"

In reply Professor Lloyd on July 16th, on letter head of the Lloyd Library, wrote Dr. Wiley as follows:

"My dear Professor Wiley:

"This morning I reach my desk after six weeks' vacation. Your letter is the first answered and I shall not delay for the stenographer. Lyman F. Kebler, Smith, Kline & French Co., Philadelphia, Pa., is the best man in America for your purpose in case he can be reached. His experience is exceptional in the line you wish an expert. His work is *unquestionable* as I know from experience of parallel investigations. He knows more to-day concerning tricks of the trade and probable adulterations than any man in the drug line and has ever aimed to enthusiastically attack adulteration and adulterators and mixers. If you can secure him you need have no time wasted in teaching or in wandering. He is expert in manipulation and conversant with the literature bearing on the subject of chemical examination.

"In my opinion he will be worth to you twice any other man and I hope his services may be secured. Should you desire details concerning Mr. Kebler's work I can furnish you data to substantiate the foregoing.

"Hoping this may reach you in time to serve you, and with regards, I am,

Sincerely yours,

JOHN URI LLOYD."

With the greatest of reluctance and for obvious reasons, Professor Lloyd's reply to Dr. Wiley's inquiry is included. It seemed to be the only course to follow. The commendations of a compatriot were of great service at the time they were given and very much appreciated, but they cannot profit me at my time of life. I am living on borrowed time.

The Secretary of Agriculture, in July 1902, requested the United States Civil Service Commission to hold an examination for securing eligibles for a chief of this laboratory. The Commission on August 16, 1902, issued the following announcement of the examination to secure eligibles for the position:

"CHIEF OF THE DRUG LABORATORY
BUREAU OF CHEMISTRY
DEPARTMENT OF AGRICULTURE
SEPTEMBER 15, 1902

"The United States Civil Service Commission announces that it is desired to establish an eligible register for the position of Chief of the Drug Laboratory, Bureau of Chemistry, Department of Agriculture. It will not be necessary for applicants to appear at any place for the examination.

"The examination will consist of the subjects mentioned below, which will be weighed as follows:

Subjects	Weights
1. Education and training	40
2. Postgraduate work and experience	40
3. (*a*) Thesis of not less than 1000 words, on the subject, 'To what therapeutic agents may State control at present be most advantageously applied.' (*b*) Scientific papers or reports of investigations published by the competitor	20
Total	100

"No one will be examined who is not a graduate in pharmacy or pharmaceutical chemistry (or an equivalent) and who has not since graduation had training and experience in the investigation of the purity and strength of substances used as therapeutic agents, and in the various sciences, a knowledge of which is essential to the successful conduct of such investigations. Experience in manufacturing pharmacy and a knowledge of commercial pharmacy will also be considered an advantage. Statements of degrees and college training must set forth the courses of study pursued and the time actually devoted to each. These statements must be certified by the appropriate officer of the school in which the work was done.

"Age limit, 20 years or over.

"From the eligibles resulting from this examination it is expected that certification will be made to the position of Chief of the Drug Laboratory, Bureau of Chemistry, Department of Agriculture, at a salary of $2000 per annum, and to other similar vacancies as they may occur.

"This examination is open to all citizens of the United States who comply with the requirements. Competitors will be rated without regard to any consideration other than the qualifications shown in their examination papers and eligibles will be certified strictly in accordance with the civil service law and rules.

"Persons who desire to compete should at once apply to the United States Civil Service Commission, Washington, D. C., for application Form 304, and special form, which should be properly executed and filed with the Commission, together with the required material, prior to the hour of closing business on September 15, 1902."

Form 304 is the application form used by the Commission for this type of examination. It is too long for even a partial inclusion in this write-up. It delves minutely into one's life history from the day of birth to the time of the examination; all kinds of work engaged in, why separated from the jobs, physical condition, chronic diseases, how freely one indulges in John Barleycorn, vouchers from two citizens as to character, use of profane language, intoxicating beverages, narcotics, etc. No one seems to know anything about the "special form" referred to, excepting that it possibly was a special form for this particular examination.

The Fiftieth Annual Meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION was held in Philadelphia, in September 1902. President H. W. Whelpley in his address called attention to the proposed investigation of drugs by the Bureau of Chemistry and that Dr. Wiley "has invited your president to outline what he considers should be the character and scope of this examination." In what manner the president complied with this request I have no knowledge but he did appoint (8) a "Committee to present the views of the AMERICAN PHARMACEUTICAL ASSOCIATION as to the most desirable character and scope of work of the new drug laboratory," consisting of A. E. Ebert, John Uri Lloyd and Lyman F. Kebler.

Professor E. L. Patch, in his report (9) as chairman of the Committee on Drug Adulteration, pointed out that the committee had received a communication from Dr. H. W. Wiley regarding the establishment of a pharmaceutical laboratory for studying the composition and adulteration of drugs. The report contains a number of recommendations and resolutions, which on motion were unanimously adopted. Two of the resolutions read: "(1) That the AMERICAN PHARMACEUTICAL ASSOCIATION offers to the Secretary of Agriculture its most cordial collaboration in this work, which promises so much benefit to the manufacturers of and dealers in drugs, as well as to the consumers thereof. (2) That this Association will use its influence with the Congress of the United States to secure a reasonable appropriation to properly carry on this work in a systematic and effective manner."

Dr. H. W. Wiley, at the first session of the Scientific Section, on invitation addressed its members (10) on the establishment of a Drug Laboratory in the Bureau of Chemistry. He outlined the nature of the work as he saw it to some extent. Said he: "Some people have the idea that the establishment of such a work as this by the Government will work a hardship on the druggist—the retailer, the wholesaler and the manufacturer. I do not think that the men in Congress who enacted this law had any such intention, and I can assure you the Secretary

of Agriculture has no such intention. On the contrary, the purpose is to help and aid, and not to annoy or embarrass. Some people have asked me, 'Do you think that Congress will continue these investigations, or will it let them drop in a little while?' Now, I have been in the public service for twenty years, and I have never yet known Congress to abandon a piece of work of this kind until it was finished, or until the end sought was attained. Congress having started this laboratory and given it an appropriation, will continue it and support it liberally. The Secretary of Agriculture has no right to come among you and select a man for chief of the new drug laboratory—that right is in the hands of the Civil Service Commission. In a short time an examination will be held for those who are willing to apply for the position of chief of the drug laboratory in the Bureau of Chemistry in the Department of Agriculture."

After listening to Dr. Wiley's address, I decided to take the examination, the final date for which was September 15, 1902. There were twenty-seven applicants. Ten passed. The rule of the Commission in such cases is to certify the three receiving the highest rating to the officer having charge of the work. I happened to be among the three and received the appointment November 26, 1902, to take office January 1, 1903. I was informed that I had received the highest rating. Due to my intimate connection with the business of my employer at the time, he asked whether my severance with the company could not be delayed for a time. To this fair request Dr. Wiley readily agreed.

The new chief reported for duty early on the morning of March 2, 1903, and found Dr. Wiley on the lower floor of the Bureau of Chemistry weighing out rations for his noted "Poison Squad," then used to test out the effects of food preservatives on digestion and health. The first official act the new chief performed was taking the oath of office, something I had never done before and it was very solemn to me. After Dr. Wiley could see me, we had a conference on the work. With a sober face he told me that Congress had authorized the establishment of the laboratory and appropriated the salary but failed to provide a place where the work could be done. He awaited the newcomer's reaction to this situation. The new chief told him that he would find a niche where he could do the work. Dr. Wiley smilingly said: "We can give you a chair, part of a desk, with reagents, chemicals and apparatus." These were the quarters I occupied for a time and I was happy in my environment. While looking over the stock of chemicals to furnish my worktable I found some of my old adulterated and mislabeled chemicals and supplies. Some of the brands of chemicals marked C.P., that I knew from previous experience, were far from being chemically pure. These findings were reported to the "Big Chief" and he immediately put me in charge of examining future chemical supplies of the Bureau, which was no small task in itself. These are some of the things found: weighted cochineal; arsenic in C.P. zinc, glycerin, sulfuric, hydrochloric and nitric acids; C.P. ether not even of U. S. P. quality; corrosive sublimate bearing a calomel label; C.P. molybdic acid eighty per cent pure; chromic acid loaded with sodium sulfate; glacial acetic acid and alcohol and the caustic hydroxides below quality claimed on the labels; potassium iodide containing sulfates and iodates; C.P. potassium cyanide testing over 100 per cent pure proved to be sodium cyanide. All these chemicals had up to this time been used for investigational work and as reagents.

At the 1903 meeting of the Association of Official Agricultural Chemists, a committee on the Testing of Chemicals was appointed, of which the Drug Chief was made chairman. In the discussion of the report of this committee in 1904, Dr. Wiley said: "The matter of the purity of reagents has been brought home to this Association by this report in a more pointed way than ever before. How many of our chemists test the purity of their reagents? Every chemist should resolve before leaving this meeting that he will not make another analysis for any work which is to be reported without assuring himself . . . . that his chemicals are sufficiently pure." Dr. Wiley was greatly shocked when he was informed regarding the quality of the chemical supply in the Bureau.

As will be recalled, there was great activity regarding the enactment of a pure food and drug law and Dr. Wiley was one of its chief protagonists. His fort was foods. The Drug Chief was instructed to give senators and congressmen every possible information and assistance in the matter of pure and adulterated drugs. The United States Pharmacopœia and the National Formulary were embodied in a number of the bills introduced in the late nineties but for various reasons the Formulary was not included in later bills and the Pharmacopœia only retained. This was strenuously objected to by many drug interests, promoters of proprietaries in particular. The result was a struggle for a more inclusive definition for drugs in the National Law. Dissatisfied drug representatives appeared at a special hearing January 20, 1903. During this hearing (11), the Omnibus drug definition was born. This definition precipitated a marked discord in the pharmaceutical profession and made plenty of demands on the Drug Laboratory, some of which are referred to in the Department of Agriculture annual reports and need not be discussed here. It should be noted here, however, that the Drug Laboratory called attention to the desirability of including the National Formulary, as a standard, in a National Food and Drug Law. Dr. Wiley was in full accord with this idea, and it was included in later Congressional bills and finally recognized in the National Law of June 30, 1906.

One day the "Big Chief" called me into his office and said: "The Bureau of Printing and Engraving has submitted a number of samples of glue and I would like to have you test them." The Drug

Chief told him that he had never tested glue and did not know anything about the subject. In reply the Boss said: "You know as much about testing glue as anyone in the Bureau." I further protested that glue was not a drug. He retorted: "Glue is certainly a drug around here and it is your job." He had shopped, without success, around the Bureau for someone to do the work and the Drug Chief was a newcomer and the logical victim. He took advantage of the definition, "A drug on the market," in this case meaning the Bureau. Some of my fellow chemists considered it a good joke. I tested the glue and did not find it such a difficult task—nothing compared with some of the knotty drug adulterations I had been called on to unravel in former years.

The glue testing represented collaboration with other branches of the Government, for which Dr. Wiley was an enthusiast. A few months later he again called me into his office, handed me a number of drug samples called "Vitality Pills," submitted by the Postmaster General for examination and report as to whether the claims made therefor were warranted. They were alleged, among other things, to contain animal extract derived from healthy bulls. Here was something novel and weird. We discussed it *pro* and *con* on numerous occasions. An examination, however, showed that these pills were of the same general composition as the commonly so-called listed aphrodisiac pills. We discussed the subject with several outstanding physicians and reached the conclusion that the claims were far-reaching and many wide of the truth and so reported to the Postmaster General. Here another thing happened. The Secretary of Agriculture did not want to sign this kind of a report. It then fell to Dr. Wiley's lot to sign and get the reports into the Solicitor's hands, which was done. The promoter was cited for a hearing, alleging the fraudulent use of the mails. After the hearing a fraud order was issued, debarring the promoter from the use of the mails, which was a signal for a royal legal battle, such as I had never seen before, but have been in many since. The case was vigorously contested, but the Government won, which meant that we would be required to do more work for the Post Office Department, in the matter of the fraudulent use of the mails. Poisons in the mails came next into the picture.

In 1903 the Drug Chief was appointed a referee on "Medicinal Plants and Drugs," in the Association of Official Agricultural Chemists. Under this appointment the methods for estimating morphine in opium were taken up and reported on for several succeeding years.

Due to the untruthful advertising and adverse publicity given proprietary medicines, either directly or indirectly, as the result of the information brought out in connection with the hearings under the various food and drug bills and otherwise, the Council on Pharmacy and Chemistry, of the American Medical Association, was organized in 1905. Dr. Wiley and I were charter members, and took a part in the work for a number of years. The Drug Laboratory made quite a few analyses of a number of proprietaries published by the Council (12), among them the acetanilid mixtures.

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(6) *Ibid.*, 30 (1899), 947.

(7) *U. S. Dept. Agric. Yearbook* (1902), pages 79 and 662.

(8) PROC. A. PH. A., 50 (1902), ix.

(9) *Ibid.*, 50 (1902), 270.

(10) *Ibid.*, 50 (1902), 276.

(11) U. S. Cong., Senate Mfg. Commit. Food Adulteration on Bill No. 3109, January 20 (1903).

(12) *J. Am. Med. Assoc.* 44 (1905), 719 and 1790.

Book Reviews

Organic Syntheses. An Annual Publication of Satisfactory Methods for the Preparation of Organic Chemicals. Volume XX. CHARLES F. H. ALLEN, Editor-in-Chief. v + 113 pages. John Wiley and Sons, Inc., 440 Fourth Ave., New York, N. Y., 1940. Price, $1.75.

This, the twentieth volume of this series, is similar in arrangement of its contents to that of preceding volumes. It contains directions for the preparation of 39 compounds involving many types of reactions. In addition, there are included for each preparation equations indicating the chemical changes involved, a detailed statement of the procedure to be followed, notes on points to which particular attention should be directed, a statement, with references, covering other methods of preparation, and illustrations of apparatus. The book is a useful reference work for anyone engaged in the preparation of organic compounds.—A. G. D.

Physical Constants of Hydrocarbons. Volume II. *Cyclanes, Cyclenes, Cyclynes, and Other Alicyclic Hydrocarbons,* by GUSTAV EGLOFF. 605 pages. Reinhold Publishing Corp., 330 West 42nd St., New York, N. Y., 1940. Price $12.00.

This book is the second volume of a four-volume work giving values for the boiling point, melting point, density and refractive index of pure hydro-

carbons. Volume I covers the paraffins, olefins, acetylenes and other aliphatic hydrocarbons. Volume II covers the alicyclic hydrocarbons, including the cyclanes, cyclenes, cyclynes and olefin- and acetylene-substituted cyclanes and cyclenes. —A.G. D.

Inorganic Chemistry (FRITZ EPHRIAM), edited by P. C. L. THORNE, M.A. (Cantab.), M.Sc., Ph.D. (London), F.I.C., and A. M. WARD, Ph.D., D.Sc. (London), F.I.C. 911 pages including index and appendix. 83 figures and several tables. Nordeman Publishing Company, Inc., 215 Fourth Ave., New York, N. Y. 3d English edition, 1939. Price, $8.00.

A great deal of information has been compiled in accordance with the general scheme used in prior editions of this book, namely, that of presenting the material in collective style rather than treating each element and related compound individually. It is stated in the introductory remarks that the collective scheme has proved to be most attractive. The whole text has been revised to modern concepts in chemistry, with portions of the book on radioactivity, isotopes and rare earths transferred from the appendix to more useful and logical places in the text.

The text is divided into six main sections which are: I. The Elements, 212 pages. II. Halogen Compounds, 174 pages. III. Oxides of Hydrogen and of the Metals, 126 pages. IV. The Compounds of Sulphur, Selenium and Tellurium, 56 pages. V. The Nitrogen, Phosphorus, Arsenic Group, 156 pages. VI. The Elements of the Fourth Group (and Boron), 110 pages. Under I, the elements, theories on the structure of matter, properties and preparation of the elements and radioactive elements and isotopes are considered. Hydrogen and oxygen compounds of the halides are treated under the Halogen Compounds with special regard to the halogen salts uni-, bi-, ter-, quadri-, quinque- and sexavalent metals. The Werner's coördination theory of halides and the ammines is treated with several illustrations for clarity. Oxides of hydrogen are treated separately, including deuterium oxide, and oxides of the metals are treated in groups under alkali, alkaline earth, rare earth, basic oxides of heavy metals and metallic oxides-acidic. Hydrogen, oxygen and halogen compounds of sulfur, selenium and tellurium are given major consideration. Under the nitrogen, phosphorus and arsenic group, the hydrides of elements of the fifth group are given considerable space. The oxygen compounds of nitrogen are given a separate chapter while those of phosphorus, arsenic, antimony and bismuth are dealt with collectively in another chapter. Sulfur and halogen compounds of the fifth group are treated in a separate chapter. Within the bounds of the sixth main section of this text, the hydrides, oxides and sulfides of the elements of the fourth group are considered along with intermetallic compounds such as carbides, silicides, borides and an introduction to alloys.—Emerson C. Beeler

Physico-Chemical Methods, by JOSEPH REILLY and WILLIAM NORMAN RAE. 3rd Edition. Volume I. Measurement and Manipulation, 686 pages. Volume II. Practical Measurements, 580 pages. D. Van Nostrand Co., 250 Fourth Ave., New York, N. Y., 1940. Price, $17.50.

The third edition of this standard work now appears in two volumes and it is expected that there will be a third volume before another edition is published.

The first volume contains a new chapter on Measures and Units by Professor O'Rahilly of Cork. Chapter IV on Observations and Calculations now includes discussions of nomography and graphical methods, applications of which are given in Chapter V.

Volume II deals with practical measurements of gases and vapors, of distillation processes, filtration, crystallization and mechanical separations. The ten chapters which follow deal with practical methods of colloid chemistry, optical methods including spectroscopy in all wave-length regions, electrochemistry and indicators, application of thermionic vacuum tubes, dielectrics and radio-activity.—A. G. D.

The Chemist at Work, by ROY I. GRADY, JOHN W. CHITTUM and others. 422 pages, 51 illustrations. Journal of Chemical Education, Easton, Penna., 1940. Price, $3.00.

This book is made up of 53 articles which have been published in the *Journal of Chemical Education*. Its purpose is to acquaint prospective students with the work of the chemist by having members of the profession discuss their jobs. Several chapters deal with the place of women in chemistry. Particular attention seems to have been given to work in experiment stations, teaching and medical laboratories, although the process industries have not been entirely overlooked. The book presents in an attractive way the varied and interesting work of chemists, and is recommended for reading by all prospective students in chemistry.—A. G. D.

Toxicity of Industrial Organic Solvents. Report No. 80 of the Medical Research Council, Industrial Health Research Board (England). Compiled by ETHEL BROWNING under the direction of the Committee on the Toxicity of Industrial Solvents. First American edition, cloth, iii + 388 pages, 5¼ x 8½. Chemical Publishing Company, New York, N. Y., 1938. Price, $3.50.

This book is a comprehensive summary of the available literature on the toxicity of the organic solvents used in the industries. It covers the entire field of organic solvents including hydrocarbons chloro compounds, alcohols, esters, ketones, cyclohexane derivatives, glycols and such other solvents as carbon bisulfide, pyridine and ethyl ether. The extent of the toxicological problems for each solvent is discussed and solutions for these problems are suggested. The book should serve as an aid to investigators working in this field.—A. G. D.